University of Alberta

Genetic divergence and conservation of butterflies of the Peace River grasslands of Canada

by

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For my parents, Lyn and Brent

Abstract

The Peace River grasslands of northern Canada are home to an isolated biota otherwise found 400 km to the south, including several butterfly species. Little is known about the genetic diversity of these populations, and much of their native habitat has been destroyed. I used mtDNA (COI) sequence data and population statistics to examine the divergence of five Peace River grassland butterfly species and five related species with broad distributions across Alberta. I compared the divergence between these two classes of species and determined that the Peace River grassland butterflies do appear to be genetically disjunct. However, the continuously distributed species suggest that a degree of genetic isolation is attributable to isolation across geographic distance. I also applied population genetic methods to a subdivided dataset and found that the characterization of genetic isolation and intermediate divergence varied a great deal depending on the length and region of DNA (within COI).

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List of Abbreviations

AMOVA – analysis of molecular variance

ANeCA – automated nested clade analysis

bp – base pairs

COI – cytochrome c oxidase subunit I

DNA – deoxyribonucleic acid

dNTPs – deoxy-nucleotide triphosphates

Fst – fixation index

GPS – global positioning satellite

kb – kilobases

mtDNA – mitochondrial DNA

NCPA – nested clade phylogeographic analysis

PAUP* – phylogenetic analysis using parsimony and other methods

PCR – polymerase chain reaction

Chapter 1: Introduction to the study region and focal taxa

1.1 Geographic location and characteristics of the Peace River grasslands

The Peace River grasslands are part of the isolated Peace River Parkland subregion of northwestern Alberta and northeastern British Columbia, Canada (Hervieux, 2002). The subregion encompasses 3120 km^2 surrounding the Peace River and its major tributaries (Natural Regions Committee 2006). The subregion, which accounts for 5% of Alberta's Parkland region, is characterized by rolling hills covered in mixed forests and dry grasslands. The northernmost extent of the subregion runs along the Peace River between the town of Peace River and the vicinity of Dunvegan. The southernmost extent encompasses an area east of Grande Prairie. The entire subregion is separated from southern Alberta and British Columbia's parkland and grassland regions by at least 300 and 400 km of boreal forest respectively (Figure 1-1).

The British Columbia side of the Peace River Parkland is not as large or well documented as the Alberta side. Moss (1952) identified an area of endemic grasslands along the Peace River leading up to Williston Lake, however most of this region was altered when the W.A.C. Bennett and Peace Canyon hydroelectric dams were built in the late 1960s (Guppy and Shepard 2001). At present, the remaining undisturbed parkland habitat is restricted to a few valleys of the Peace River and tributaries, and a limited number of upland sites (Guppy and Shepard 2001, Stamford and Taylor 2005).

Approximately 70% of the Peace River Parkland is cultivated, including virtually all of the upland sites. A large but unquantified proportion of the river valley and bank grassland is undisturbed (most north-facing slopes are covered in mixed woods), but only 0.5% of the upland grassland sites are intact. Remaining Peace River grassland sites are extremely fragmented, and are now primarily restricted to steep river valleys and a small preserve near Grande Prairie known as Kleskun Hill (Hervieux 2002, Natural Regions Committee 2006). Some semi-native rangeland along the Peace River is used by cattle farming operations.

1.2 Endemism in the Peace River grasslands

The Holocene Hypsithermal warming trend that occurred between 6000 and 9000 years ago led to the expansion of grassland across much of Alberta (Strong and Hills 2003). A subsequent climatic cooling period allowed boreal forest to encroach upon much of this grassland, which in turn led to the disjunction of the Peace River Parkland and its biota. A number of plants and animals that are isolated in the Peace River region are otherwise only found in grassland and parkland 400-500 km to the south (Kondla et al. 1994, Strong and Hills 2003, Natural Regions Committee 2006).

The Peace River grasslands were first described by 19th century explorers and settlers. Agricultural settlement began in the early twentieth century and expanded so rapidly that the former extent of the grasslands can only be inferred from the region's soil types (Natural Regions Committee 2006). Despite the extreme loss or conversion of habitat, a number of typically southern grassland species remain

associated with the Peace River region. Moss (1952) and Strong and Hills (2003) have noted many disjunct plants, including grasses and cactus. Fur-trade records indicate that pronghorn antelope (*Antelocarpa americana* [Ord]) were present in the Peace River region until the mid 19th century (Ferguson 2003). Acorn (2001) has noted several species of tiger beetle (Cicindellidae) with disjunct distributions in the Peace River region, while Schmidt (2007) has identified several disjunct tiger moths (Noctuidae: Arctiinae). However, no faunal group restricted to the region has been as well documented as the Peace River butterflies and skippers.

Early checklists of the butterflies of Alberta and British Columbia first documented some of the diversity in the Peace River region (Bowman 1951, Llewellyn-Jones 1951). Case and Bird (1977) extended the known diversity considerably, though the most thorough treatment of the regional butterfly fauna was conducted by Kondla et al. (1994). That study noted the following ten species with Peace River populations that were discontinuous from southern Alberta (i.e. absent from the intervening 300-400 km of boreal forest): *Pyrgus communis* (Grote), *Oarisma garita* (Reakirt), *Hesperia assiniboia* (=*comma assiniboia*) (Lyman), *Papilio machaon pikei* Sperling, *Satyrium* (=*Harkenclenus*) *titus* (Fabricius), *Satyrium liparops* (Le Conte), *Chlosyne gorgone* (Hübner), *Chlosyne palla* (Boisduval), *Oeneis uhleri* (Reakirt) and *Oeneis alberta* Elwes (Kondla et al. 1994). Hervieux (2002) further assessed the distribution and abundance of eight of these butterflies (all but *P. communis* and *O. garita*).

All of the disjunct Peace River grassland species can be classified as sensitive in the Peace River region (Hervieux 2002), and provincially sensitive in British

Columbia (Guppy and Shepard 2001). Both Kondla et al. (1994) and Hervieux (2002) have stressed the importance of the Peace River butterfly fauna, and have suggested that steps be taken to better classify and understand them.

1.3 Study design

1.3.1 Sampling design

Butterflies were collected at a number of locations in the Peace River region and in areas of southern Alberta and adjacent areas in order to facilitate comparisons between the disjunct and contiguous populations. For a detailed treatment of the specimens and localities, see Appendix 1 and Chapter 2. Between 12 and 25 specimens were extracted per species per region. At least 1420 base pairs of cytochrome c oxidase subunit I (COI) DNA were subsequently sequenced for each specimen sampled, giving 28 to 45 specimens per species. Whenever possible, equal numbers of specimens were sequenced from each region, though the availability of specimens was limited in some taxa. Each disjunct species was partnered with a continuously distributed butterfly from the same family (and in one case, genus).

1.3.2 Focal taxa

My study focuses on five of the butterfly species that have isolated Peace River populations: *O. uhleri*, *O. alberta*, *S. titus*, *S. liparops*, and *P. machaon pikei*

(*P. machaon dodi* McDunnough in southern Alberta). These species were chosen for several reasons. First, they are all closely associated with native prairie. Second, they are all univoltine (with the exception of *Papilio machaon dodi*, which is facultatively bivoltine, Sperling 1987, Bird et al. 1995). Finally, thanks to the work of a number of collectors, there were already moderate numbers of samples available for molecular analysis.

The disjunct grassland butterflies were partnered with common butterflies that have continuous distributions. The five continuously distributed species are (respective to the disjunct species listed above): *Coenonympha tullia* (Müller), *Erebia epipsodea* Butler, *Glaucopsyche lygdamus* (Doubleday), *Plebejus saepiolus* (Boisduval), and *Papilio canadensis* Rothschild and Jordan. These species are common throughout meadows, disturbed grassy areas, and mixed forest in much of Alberta, and were not expected to exhibit any population structure related to range discontinuity.

1.4 Species accounts

All nomenclature follows Layberry et al. (1998), although some alternate taxonomic designations are discussed below. Subspecies names are primarily mentioned in the context of southern Alberta; with the exception of *Papilio machaon pikei*, the subspecies status of the disjunct Peace River populations is uncertain (Kondla et al. 1994, Bird et al. 1995). Figure 1-2 illustrates all ten species discussed below.

1.4.1 Disjunct species

Family Nymphalidae, subfamily Satyrinae

Alberta Arctic

Oeneis alberta Elwes 1893

This medium-sized (34-43 mm wingspan) greyish brown butterfly has zero to four eyespots on both sides of the forewing, and zero to two eyespots on both sides of the hindwing (Layberry et al. 1998). Males tend to patrol or hilltop, with butterflies congregating on broad grassy areas. Eggs are laid on grass blades; fescue (*Festuca* spp.) and other grasses have been recorded as host plants. This butterfly overwinters as a prepupal larvae and adults emerge very early in the year (mid April), flying for one to one-and-a-half months (Bird et al. 1995). The Peace River populations of this butterfly represent the northernmost edge of the range. The species is found south through the Rocky Mountains to the southern United States, and east to Manitoba (Layberry et al. 1998). All Canadian populations are believed to represent the nominate subspecies *Oeneis alberta alberta* (Layberry et al. 1998). Peace River specimens tend to be darker and more heavily marked (Bird et al. 1995).

Uhler's Arctic

Oeneis uhleri (Reakirt 1866)

This species is a medium-sized (35-46 mm wingspan) greyish brown butterfly with up to five eyespots on the ventral hindwing, though these may be quite small (Layberry et al. 1998). Males patrol, and mating or chasing pairs can be abundant on

hilltops in the mid-afternoon (Bird et al. 1995, pers. obs.). Eggs are laid on grass blades, most likely on *Festuca* and *Stipa* species. Larvae overwinter and adults tend to emerge in mid-May and fly until early June. There are also alpine populations that emerge in early August (Bird et al. 1995). There are three subspecies recognized in Canada; *Oeneis uhleri varuna* is present throughout southern Alberta. The status of the Peace River population in relation to two northern subspecies (*O. uhleri nahanni* from the Northwest Territories and *O. uhleri cairnesi* in the Yukon) is uncertain. Kondla et al. (1994) noted that the Peace River populations tend to be darker and possess more eyespots than their southern counterparts. The range of *O. uhleri* extends into northern Canada, south to New Mexico, and east through the prairies to southern Manitoba (Layberry et al. 1995, Guppy and Shepard 2001).

Family Lycaenidae, subfamily Theclinae

Coral Hairstreak

Satyrium (=*Harkenclenus*) *titus* (Fabricius 1793)

This small brown butterfly (25-32 mm wingspan) has a weak band of black dots and a submarginal band of coral spots (though these may be obscured in damaged or worn individuals). Females are larger, and hindwing shape is sexually dimorphic. (Bird et al. 1995, Layberry et al. 1998). Eggs are laid on *Prunus* spp. (especially Choke Cherry) and *Amelanchier alnifolia* (Saskatoon) at the edges of native grasslands, hatching the following spring. Adults emerge in June or July, but peak flight time is July-August (Bird et al. 1995, Layberry et al. 1998). Adults can be secretive, and tend to perch within clusters of their host plant, although they will

hilltop and nectar in the mid afternoon (Kondla et al. 1994; pers. obs.). This species ranges widely across North America, east to Newfoundland and south to California and Georgia. The Peace River population represents the northernmost range extent of the species (Layberry et al. 1995, Shepard and Guppy 2001). The subspecies status of the southern Alberta population is uncertain; Bird et al. (1995) considered it to be the prairie subspecies *Satyrium titus immaculosus*, while Guppy and Shepard (2001) believed it to be part of the nominate race *S. titus titus*. Peace River specimens are darker than those found in southern Alberta, which in turn, are darker and less heavily spotted than populations from eastern Canada (Kondla et al. 1994).

Striped Hairstreak

Satyrium liparops (Le Conte 1833)

This small greyish brown butterfly (25-35 mm) has two small tails on the hindwing bounded by a small orange dot or band. The ventral hindwing is also covered in small broken black and white lines (Bird et al. 1995, Layberry et al. 1998). Eggs are laid on *Prunus* spp. and *Amelanchier alnifolia* (Saskatoon) at the edges of native prairie, hatching the following spring. Adults emerge in June; peak flight time is July-August (Bird et al. 1995, Layberry et al. 1998). Adults can be very secretive and difficult to collect, though they can be flushed from large clusters of Saskatoon. This species is at its northernmost extent in the Peace River region, and is otherwise found in southern Canada east of the Rockies south throughout the United States. The subspecific status of Alberta specimens is unclear; Guppy and Shepard (2001) identify Peace River specimens as part of the widespread subspecies *Satyrium liparops fletcheri* and the southern Alberta specimens as *S. liparops aliparops*.

However, Kondla et al. (1994) note that the Peace River populations are darker and more clearly marked than any other Canadian specimens.

Family Papilionidae, subfamily Papilioninae

Old World Swallowtail

Papilio machaon Linnaeus 1758

Papilio machaon dodi McDunnough 1939

Papilio machaon pikei Sperling 1987

Papilio machaon oregonius Scudder 1869

Papilio machaon aliaska Edwards 1876

Papilio machaon is a large black and yellow butterfly (51-95 mm wingspan) with a prominent tail and orange and blue eyespots on both sides of the hindwing (Layberry et al. 1998). The Peace River subspecies *P. machaon pikei* can be distinguished from southern Alberta populations of the species by the amount of yellow hair on the bottom of the thorax, and by its geographic range. The southern Alberta subspecies *P. machaon dodi* lacks yellow thoracic hair in the first generation. Eggs are laid on Dragonwort (*Artemisia dracunculus*) where the larvae feed before overwintering as pupae. *Papilio m. dodi* flies from early May to August, with a dip in numbers in mid June before a partial second generation emerges in late July. *Papilio m. pikei* has been recorded from early June to July (Bird et al. 1995). *Papilio machaon* is part of a complex and variable species group with many described subspecies across North America and Eurasia. Two other subspecies were examined in this study: *Papilio machaon aliaska* Edwards from Alaska, the Yukon, and

northern British Columbia and *Papilio machaon oregonius* Scudder from southern British Columbia and the Pacific Northwest. An additional subspecies, *Papilio machaon hudsonianus*, has been reported from boreal Alberta, though it is infrequently encountered. Further complicating matters, *Papilio machaon* forms a hybrid zone with *Papilio zelicaon* in southwestern Alberta (Sperling 1987, Bird et al. 1995). Nonetheless, combinations of morphological features generally differentiate the Canadian subspecies (Sperling 1987).

1.4.2 Continuously distributed species

Family Nymphalidae, Subfamily Satyrinae

Common Ringlet

Coenonympha tullia (Müller 1764)

This is a relatively small to medium-sized orange-brown to grey butterfly (27-39 mm wingspan), with or without eyespots on the ventral forewing (Layberry et al. 1998). Eggs are laid on a variety of grasses where larvae feed before overwintering. Adults emerge in late May and fly throughout the summer. There may be a partial second generation in many parts of the range of this species (Bird et al. 1995, Layberry 1998). This is a complex species, with numerous subspecies described in Canada (Layberry et al. 1998). The total range of this species group is considerable, spanning northern Eurasia, as well as the northern half of North America (Layberry et al. 1998, Guppy and Shepard 2001). Kondla et al. (1994) noted that the Peace River populations found in native grasslands tend to be darker than those from roadsides in

the same region or parts of southern Alberta. Guppy and Shepard (2001) treated the Peace River, southern Alberta, and intervening populations as the subspecies *C. tullia benjamini*. There is considerable morphological variation seen in this species group.

Common Alpine

Erebia epipsodea Butler 1868

This species is a medium sized dark brown butterfly (34-45 mm wingspan) with orange-ringed black eyespots on both sides of the forewing (Layberry et al. 1998). Eggs are laid on a variety of grasses where the larvae feed before overwintering. Adults emerge in May and can be found in wet meadows and mixed woody areas throughout the summer (Bird et al. 1995, Layberry et al. 1998). This butterfly ranges from Alaska south to Oregon, through the Rocky Mountains to New Mexico, and east to Saskatchewan (Guppy and Shepard 2001). There are two subspecies tentatively recognized in Alberta (*E. epipsodea epipsodea* in the mountains and foothills, and *E. epipsodea freemani* in the rest of the province), however most specimens in the province are considered intergrades (Bird et al. 1995). Guppy and Shepard (2001) alternately designate the non-montane populations (including Peace River and north-central British Columbia) as part of the subspecies *E. epipsodea sineocellata*. They also recognize the subspecies *E. epipsodea remingtoni* in the Yukon and northwestern parts of British Columbia.

Family Lycaenidae, Subfamily Polyommatinae

Silvery Blue

Glaucopsyche lygdamus (Doubleday 1841)

This small butterfly (18-28 mm wingspan) possesses a powdery metallic-blue upper wing bordered by dark grey (widest in the females) (Layberry et al. 1998). The ventral wings are light grey with a row of white-ringed black spots. Eggs are laid on a variety of legumes where larvae feed before overwintering as pupae. Adults emerge in April and fly throughout the summer, though the peak flight period is in June and July (Bird et al. 1995). This species is found across Canada, south through the Pacific states, and in some portions of the eastern United States (Layberry et al. 1998, Guppy and Shepard 2001). Layberry et al. (1998) only recognize a single subspecies in Canada, *G. lygdamus couperi*, though Guppy and Shepard (2001) call the populations west of the Rockies *G. lygdamus columbia*.

Greenish Blue

Plebejus saepiolus (Boisduval 1852)

This species is a small, common butterfly (21-28 mm wingspan). Males possess a metallic-blue upperwing bordered by white, while females are brown above with a bluish tinge near the body. The ventral wing surface of both sexes is covered in two rows of white-ringed black spots and possesses several small orange spots near the apex (more pronounced in the female) (Bird et al. 1995, Layberry et al. 1998). Eggs are laid on various clovers (*Trifolium* spp.) where the larvae feed on developing flowers before overwintering. Adults emerge in mid-May in the south, later in the north. Adults fly throughout the summer. There are two Canadian subspecies, one of which is confined to Vancouver Island and may be extinct (*Plebejus saepiolus insulana*). The remaining subspecies, *Plebejus saepiolus amica*, is found throughout

the rest of Canada, the western United States, and the Great Lakes region (Layberry et al. 1998, Guppy and Shepard 2001).

Family Papilionidae, Subfamily Papilioninae

Canadian Tiger Swallowtail

Papilio canadensis Rothschild & Jordan 1906

This is a large yellow and black butterfly (70-100 mm wingspan) whose hindwings possess a broad black band along the inner edge and a single tail (Bird et al. 1995, Layberry et al. 1998). Eggs are laid on a variety of trees where the large green larvae feed and pupate before overwintering (Bird et al. 1995, Guppy and Shepard 2001). Adults emerge in May and fly throughout the summer. This species ranges broadly across Canada east of the Rocky Mountains, extending into parts of the northern United States (Layberry et al. 1998, Guppy and Shepard 2001). There are no described subspecies (Layberry et al. 1998), however it is known to hybridize with *Papilio rutulus* in southern British Columbia (Guppy and Shepard 2001) and *Papilio glaucus* in the Great Lakes region (Stump et al. 2003). This species, along with *Papilio rutulus* and *Papilio glaucus*, was formerly considered a single widespread species.

1.5 Significance and Research Overview

The Peace River butterflies provide an excellent opportunity to study the conservation and evolutionary significance of recently isolated populations. Chapter

2 focuses on mitochondrial DNA (COI) sequence divergence of these species in the context of a group of related but widely distributed 'control' species that have been grouped with the species with isolated populations. These 'control' species are common in the Peace River region but do not exhibit disjunct distributions (Kondla et al. 1994); instead they are continuously distributed south (and in two cases, also north) of the region. By comparing the level of divergence in the continuously distributed and disjunct species I am able to address two questions with important practical sequences; 1) do single species studies provide a consistent and accurate characterization of general patterns of disjunction in areas of endemism, and 2) can isolation due to geographic disjunction be separated from isolation across geographic distance. Both of these issues are crucial to determining the conservation significance of divergent and/or relictual populations.

Chapter 3 examines whether the robustness of evolutionary inferences based on mitochondrial COI sequences depends on the lengths and locations of those DNA sequences. The 1420 bp sequences used in Chapter 2 were divided into smaller segments (halves, thirds and quarters) and analysed using the kinds of population genetic and phylogeographic tests that are commonly employed in conservation genetic studies. A recently designed framework for characterizing intermediate polyphyly (Omland et al. 2006) was also applied to the dataset. These measures allowed me to address whether a specific size of sequence and/or particular subregion of COI is best suited to characterizing the divergence of isolated populations, thereby contributing to the ongoing evaluation of DNA barcoding (Hebert et al. 2003a, b) and the characterization of mitochondrial divergences below the species level.

We are running out of time to study the Peace River grasslands and their isolated fauna, as agriculture has left the region badly fragmented and diminished in extent. Documentation of the region's butterflies would lend credence to initiatives being undertaken to protect the Peace River's threatened grassland habitat. This issue is also important beyond the scope of Peace River and grassland Lepidoptera; Gauthier and Wiken (2003) estimate that only 25-30% of Canada's native grasslands are intact, and only 3.5% of that is protected. This land has largely been set aside to protect endangered mammals, and very little is known about that status of Canada's native grassland invertebrates. This research may contribute to establishing evidence-based protected areas and/or protective legislation for endangered taxa. This research also provides baseline information that may serve as a foundation for future studies on butterfly range shifts occurring due to climate change. This is particularly relevant given the susceptibility to extinction of isolated populations, such as those found in habitat pockets (Collinge 2000, McLaughlin *et al.* 2002).

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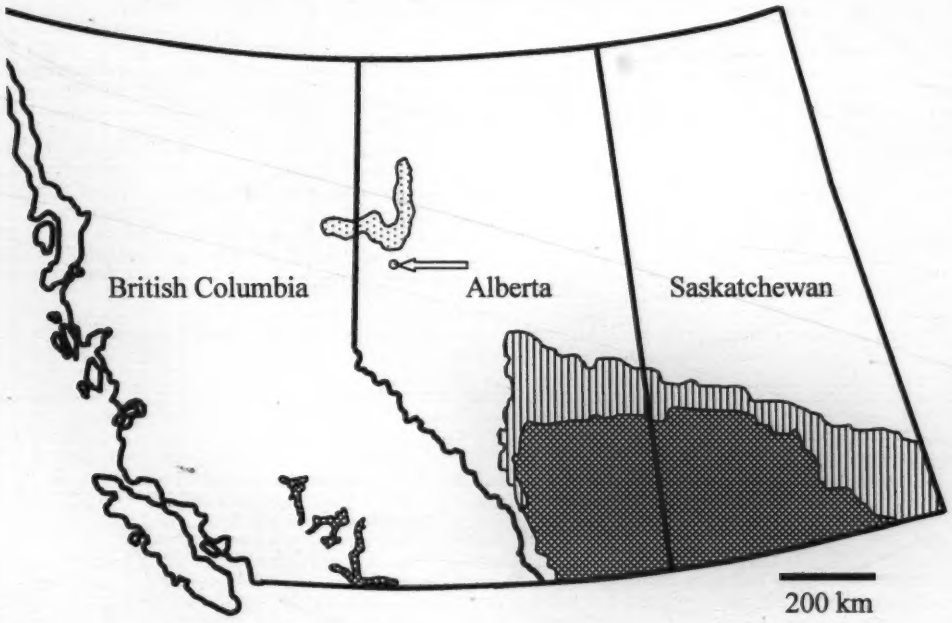
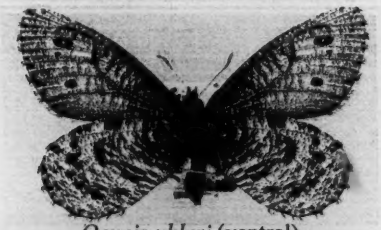
Figure 1-1. Distribution of grassland and parkland in Western Canada



The Peace River grasslands straddle the Alberta-BC border and are shaded with dots. Southern grasslands are crosshatched while parkland is shaded with vertical lines. Kleskun Hill Natural Area is indicated with an arrow. Modified after Moss (1952), Gayton (2003) and Natural Regions Committee (2006).

Figure 1-2. Illustrations of the study species. Scale varies between species.

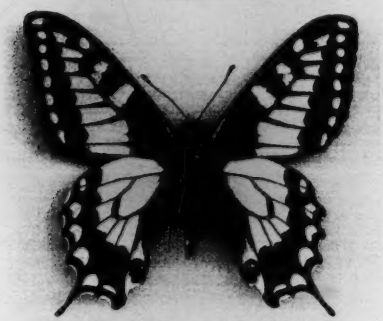


Oeneis uhleri (ventral)

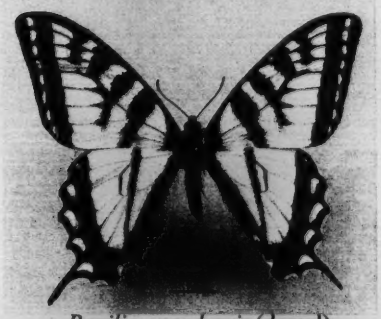
Oeneis alberta (ventral)

Satyrium titus (ventral)

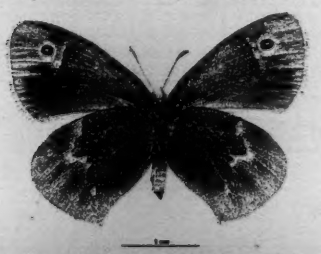
Satyrium liparops (ventral)



Papilio machaon pikei (dorsal)

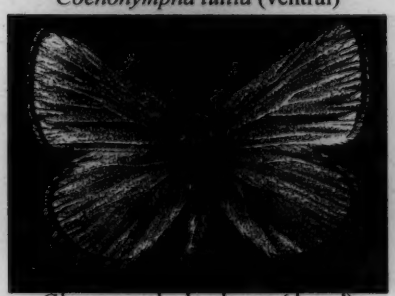


Papilio canadensis (dorsal)



Coenonympha tullia (ventral)

Erebia epipsodea (dorsal)



Glaucopsyche lygdamus (dorsal)

Plebejus saepiolus (dorsal)

All images taken by Gary Anweiler. Copyright University of Alberta Strickland Museum

Chapter 2 - GENETIC DIVERGENCE DUE TO DISJUNCTION VERSUS GEOGRAPHIC DISTANCE IN PEACE RIVER GRASSLAND BUTTERFLIES

2.1 Introduction

Discontinuously distributed habitats are often home to an endemic biota whose isolation can elucidate many facets of evolution and biogeography (Knowles and Carstens 2007). Phylogeography has been very important for characterizing diversification of these endemic organisms (Anderson 1949, Avise 1996, Hewitt 1996, Soltis *et al.* 2006). However, many of the inferences that emerge from individual studies have limited congruence among co-distributed species (Lambeck 1997, Roberge and Angelstam 2004, Soltis *et al.* 2006). Factors such as variable extent of genetic diversity at the time of isolation, gene flow within populations, and mutation rates can potentially confound phylogeographic inferences (Funk and Omland 2003, Payne *et al.* 2005). This has been reinforced by numerous phylogeographic meta-analyses demonstrating genetic variability between species with similar current distributions (Thompson 1999, Wen 1999, Gillespie and Roderick 2002, Soltis *et al.* 2006, Sommer and Nadachowski 2006). Moreover, characterizations for single species may fail to recognize true population divergence due to factors such as introgression and hybridization (see Nice *et al.* 2002, Rubinoff and Sperling 2004, Gompert *et al.* 2006, for examples from Lepidoptera). Despite these findings, most phylogeographic studies (including those making conservation recommendations) continue to focus on single species or species groups. Based on a

survey of 249 phylogeographic and population genetic studies published over two years in Molecular Ecology (January 2006-August 2007), 85% of studies dealt with single populations, species, or species groups, 5% dealt with multiple species within a genus, and only 10% dealt with multiple species in different genera (Bromilow 2007 Chapter 4).

Comparisons of multiple species with disjunct distributions are only one way to understand biotic divergence. Analyses of widespread species provide an important window into understanding divergence in more discontinuously distributed species (Ellis *et al.* 2006, Whitely *et al.* 2006, Pearman and Weber 2007), although the development of this approach is still in its early stages. My literature survey found only five studies in which common comparative species were used to help characterize genetic divergence, and only two of these examined multiple focal taxa (Bromilow 2007 Chapter 4). To date, no studies have suggested a consistent methodological approach to distinguishing genetic isolation by disjunction from isolation by geographic distance. Common species with continuous distributions can act as controls, identifying the degree of isolation due solely to geographic distance in a given region.

The Peace River grasslands of Alberta and British Columbia constitute a unique habitat subregion, isolated from other grasslands by 300-400 kilometres of boreal and aspen forest (Natural Regions Committee 2006). This northern grassland area was isolated due to temperature changes that occurred 6000 to 9000 years ago (the Holocene Hypsithermal, Strong and Hills 2003), and is home to a biotic assemblage that is most similar to the grasslands of southern Alberta and British Columbia (see

for example Acorn 2001, Ferguson 2003, Strong and Hills 2003). Morphologically and genetically distinct populations of Peace River grassland organisms (including Lepidoptera: Sperling 1987, Kondla *et al.* 1994), provide an excellent opportunity to study more general processes in biodiversity generation. The hypsithermal warming trend that first created the Peace River grasslands may currently be echoed by anthropogenic climate change, and thus, these grasslands may constitute model systems that provide crucial insight into the conservation of changing habitats. However, upland native grasslands now only exist in only 0.5% of their former range within the Peace River (Hervieux 2002), and the native plants and animals of this region are in serious danger of being lost.

Insects are excellent indicators of endemism, as even weak habitat barriers can provide near complete interruption of gene flow in many species (Thomas 2005). Butterflies in particular have proven useful as conservation foci; they have high niche specificity, are relatively well studied, and even uncommon species can be locally abundant (Matter *et al.* 2003, Thomas 2005).

Peace River grassland butterflies have been discussed by several authors, all of whom have noted some morphological or genetic divergence between these populations and other populations of the same species (see Case and Bird 1977, Sperling 1987, Kondla *et al.* 1994, Bird *et al.* 1995, Hervieux 2002). I selected five grassland-restricted species with disjunct distributions in the Peace River region and southern Alberta (*Oeneis uhleri* (Reakirt), *O. alberta* Elwes, *Satyrium titus* (Fabricius), *S. liparops* (Le Conte), and *Papilio machaon* Linnaeus) and matched them with five species that are continuously distributed across Alberta

(*Coenonympha tullia* (Müller), *Erebia epipsodea* Butler, *Glaucopsyche lygdamus* (Doubleday), *Plebejus saepiolus* (Boisduval), and *Papilio canadensis* Rothschild and Jordan).

Analyzing multiple disjunct species as well as multiple continuously distributed species affords the opportunity to address two fundamental biogeographic and conservation problems. First, this approach allows an assessment of whether the population structure of a single discontinuously distributed species can serve as an adequate characterization of biogeographic patterns in other species with similar distributions. Second, the inclusion of continuously distributed species allows divergence by disjunction to be distinguished from divergence due solely to geographic distance. A clearer understanding of these factors should contribute to more consistent ranking of conservation priorities, as well as a better understanding of the effects of climate change on isolated populations.

2.2 Materials and methods

2.2.1 Taxonomic sampling

The five continuously distributed species are relatively closely related to their disjunct 'partner' species, however they were not chosen for strictly paired analyses, but rather to avoid any stochasticity that might arise from examining broadly unrelated species. *Oeneis uhleri* and *O. alberta* (Nymphalidae, Satyrinae) were paired with two continuously distributed satyrine butterflies, *Coenonympha tullia* and

Erebia epipsodea. *Satyrium* (=*Harkenclenus*) *titus* and *Satyrium liparops* are both hairstreak butterflies (Lycaenidae, Theclinae). There are no hairstreaks with continuous distributions across Alberta, so two blues (Lycaenidae, Polyommatinae) were chosen as comparisons: *Glaucopsyche lygdamus* and *Plebejus saepiolus*. The final disjunct species was the only butterfly that had previously been described as a unique taxonomic entity endemic to the Peace River region. The Old World Swallowtail *Papilio machaon* is divided into several subspecies, of which *Papilio machaon pikei* Sperling is restricted to the Peace River region, while *P. machaon dodi* McDunnough is found in southern Alberta and southward in the Great Plains. I also examined the subspecies *P. machaon aliaska* Edwards (found in alpine habitats of Alaska and northern British Columbia) and *P. machaon oregonius* Scudder (found in interior grasslands of southern British Columbia and the northwest United States) in some analyses. The *machaon* complex was compared to the Canadian Tiger Swallowtail, *Papilio canadensis*, which is in a separate subgenus. The five continuously distributed species are all common in Alberta and are associated with a wide range of grasslands, parkland, and mixed forest habitat. The five disjunct species are all found in native or near-native grassland (Bird *et al.* 1995). All ten species are primarily univoltine, with the exception being the subspecies *Papilio machaon dodi*, which is partially bivoltine (Sperling 1987).

Collections of the ten species were made from 78 localities, predominately from Alberta, Canada (Figs. 2-1, 2-2, Table 2-1). Adult butterflies were hand collected between 1996-2006 by the authors and collaborators; however the bulk of

the specimens were collected between May 2005 and August 2006. Specimens were killed by freezing, or in some cases air-dried.

2.2.2 Molecular techniques

Whole genomic DNA was extracted from 347 butterflies using the QiaAMP DNA Mini Kit and eluted in 100-200 µl of Buffer AE (Qiagen, Valencia, CA) (Figs. 2-1, 2-2 and Appendix 1). For each species, between 12 and 25 specimens were extracted for each of two regions (Peace River area vs. southern Alberta and southward). Corresponding vouchers were assigned UASM identification numbers and deposited in the Strickland Museum of Entomology (University of Alberta). Whenever possible, equal numbers of specimens were extracted from both southern Alberta and the Peace River region, although this was limited by specimen availability. Seven butterflies had been previously extracted and/or sequenced for other DNA-based studies (Caterino and Sperling 1999, Caterino *et al.* 2001, Zakharov *et al.* 2004, Pena *et al.* 2006). Extracted DNA was stored at –20°C prior to amplification by polymerase chain reaction (PCR).

A region of the mitochondrial gene cytochrome *c* oxidase subunit I was amplified corresponding to nucleotide positions 1534-2954 of *Drosophila yakuba* mtDNA). Most PCRs were carried out in a reaction solution consisting of 35.5 µl double-distilled water (Millipore Corp., Billerica, MA), 5 µl of 10 X buffer (Qiagen) 2 µl of 25 nM $MgCl_2$ (Qiagen), 1 µl of 10 mM dNTPs (Roche Diagnostics, Indianapolis, IN), 2 µl each of two 5pM/µl heterologous primers, 0.5 µl

(approximately 2.5 units) of Taq polymerase and 2 µl of DNA. Whenever possible, PCRs were conducted to amplify two overlapping fragments of about 750 bp each (Lyn-K525, Jerry-Pat2) (Table 2-2). When amplification of these fragments was impossible, several internal primers were used. PCRs were performed in a T-Gradient PCR Thermal Cycler (Biometra, Göttingen, Germany) using the following program: an initial denaturation for 2 minutes at 94°C; 34 cycles consisting of denaturation for 30s at 94°C, annealing for 30s at 45°C, and extension for 2 min at 72°C; followed by a final extension at 72°C for 5 minutes. Products obtained through PCR were visualized on agarose gel to ensure correct fragment size and quality. Fragments were cleaned using the QIAquik PCR purification kit (Qiagen).

Forward and reverse strands were sequenced using the same sets of primers as the initial PCR amplification. Sequencing reaction solutions consisted of 1.5 µl of double-distilled water (Millipore), 3 µl of 2.5 X sequencing buffer, 1 µl of BigDye terminator cycle sequencing mix (PE Applied Biosystems, Foster City, CA), 0.5 µl of one of the previously listed primers, and 4 µl of purified PCR product. The reaction program consisted of the following: an initial 1 minute step at 96°C, and 29 cycles of 96°C for 10s, 50°C for 5s, and 60°C for 4 min. Sequencing products were cleaned using Sephadex G-50 fine packed columns (Amersham Biosciences Inc., Piscataway, NJ) or NaAC-EDTA ethanol precipitation (Big Dye protocol, PE Applied Biosystems). Clean sequencing product was visualized on either an ABI 377 automated DNA sequencer or an ABI 3730 automated capillary sequencer (PE Applied Biosystems).

Construction and verification of contiguous sequences were performed in Sequencher 4.1 (Gene Codes Corp. 2001). At least 1420 bp of sequence was obtained for each specimen. No insertions or deletions were found within or between species, so sequences were aligned manually in PAUP* version 4.0 beta 10 (Swofford 2003).

2.2.3 Population genetic and phylogenetic analyses

Phylogenetic reconstructions for preliminary assessments of haplotype relationships were obtained using unweighted parsimony in PAUP* 4.0b10 (Swofford 2003). Any variable nucleotide positions were treated as unordered characters. A single state was assigned to each variable nucleotide or character. Missing data were assigned as Ns (only applicable to two *Plebejus saepiolus* sequences). I used heuristic searches with 1000 random-addition replicates, using TBR branch swapping.

Nested clade phylogeographic analysis (NCPA) was conducted for all species using Automated Nested Clade Analysis (ANeCA v 1.0 Panchal 2007, Panchal and Beaumont 2007). With the exception of *P. machaon*, all species were categorized into northern (Peace River) and southern populations. *Papilio machaon* was divided into subspecies, with *P.m. pikei* and *P.m. dodi* corresponding to Peace River and southern Alberta localities respectively. Additional subspecies from outside of Alberta (*aliaska*, and *oregonius*) were assigned to their respective geographic regions and treated as populations. I found four *P. machaon* haplotypes that were highly

divergent, and three of these were too genetically distinct to be analysed in NCPA (i.e. too many theoretical steps to link the populations). These individuals were excluded from NCPA. Likewise, a highly divergent haplotype lineage of *C. tullia* could not be analysed in NCPA, and was excluded (see Results and Discussion). NCPA was conducted on the remaining sets of both *P. machaon* and *C. tullia*. Haplotype networks were constructed with 95% confidence limits using the TCS function of ANeCA (Clement *et al.* 2004). Reticulations were left unresolved. Clade nesting was performed using the nesting algorithm in ANeCA (Panchal 2007, Panchal and Beaumont 2007). The GeoDis (Posada *et al.* 2000) function of ANeCA was used to calculate the statistical significance of population genetic distances, and results were analysed using the automated GeoDis inference key. Automated inference key results were checked manually against the most recent GeoDis inference key to ensure accuracy and to safeguard against sampling inadequacy (November 11 2005 key). All geographic coordinates were determined in the field at the time of collecting or post-hoc using Google Earth (2007) (Table 2-1).

AMOVA (Excoffier *et al.* 1992) and related statistics (Variance components, Fst, and m-values), molecular diversity (haplotype diversity and percent sequence divergence), and exact tests of sample differentiation were calculated for all species using Arlequin 2.0 (Schneider *et al.* 2000). Haplotype diversity was calculated from the frequency of haplotypes as a proportion of the total (Nei 1987). Percent sequence divergence is determined as the proportion of bases that vary between all given haplotypes. Standard deviations for both values were determined by using parametric bootstrapping methods. The M-value (absolute value of migrants between

populations per generation) was estimated as M=(1-Fst)/2Fst (Schneider *et al.* 2000). These statistics were used to determine genetic variation within and between Peace River and southern Alberta butterfly populations. Populations were grouped in the same manner as for NCPA, however the anomalous haplotypes of *P. machaon* and *C. tullia* were included and excluded in separate analyses.

Statistical correlation between genetic and geographic distances was examined using Mantel tests (Manly 1994) performed in Isolation by Distance Web Service version 3.11 (IBDWS) (Jensen *et al.* 2005). Populations were grouped by individual locality, and average genetic distance for any given locality was taken from the mean of all distances. Only the Alberta populations of *P. machaon* were examined. Analyses were conducted for *P. machaon* and *C. tullia* with and without the divergent haplotypes.

2.3 Results

Maximum parsimony phylograms for eight of ten species were relatively simple (not shown) and relationships could be adequately summarized in NCPA networks (see below). However, those of *Papilio machaon* and *Coenonympha tullia* are shown in Figures 2-3 and 2-4, since each of these species had divergent haplotypes that could not be included in NCPA. The *P. machaon* tree includes a specimen of *P. machaon gorganus* from France, which was very similar to the common North American haplotypes. The four divergent haplotypes (including one divergent *pikei* haplotype included in the nested clade analyses) are substantially

different from all other haplotypes examined. Likewise, they are not any more closely related to other subspecies of *Papilio machaon* (the most distinct of which is used as an outgroup in Figure 2-3). The divergent haplotype included in NCPA exhibited approximately 1% divergence from the other more common haplotypes, while the remaining anomalous sequences were diverged by 1.5-2%.

The *C. tullia* tree also includes a specimen from California and a group of four identical *C. nipisiquit* from New Brunswick. The highly divergent Peace River haplotype (from seven individuals) is considerably more divergent than either of these two populations, exhibiting approximately 3% COI sequence divergence from the other haplotypes.

The divergent haplotypes for both species were resequenced for all specimens to ensure that contamination or other error had not occurred. One *C. tullia* specimens was re-extracted and resequenced. No evidence of contamination was found.

Statistical summaries of genetic diversity showed variation across both disjunct and continuously distributed species (Table 2-3). All five disjunct species exhibited greater haplotypic diversity and % sequence divergence in the south than in the Peace River region except when the divergent haplotypes of *P. m. pikei* were included. The continuously distributed species exhibited more similar genetic variation between regions, though only *P. saepiolus* had a higher number of haplotypes in the Peace River region. Haplotype diversity was greater in the Peace River region for two species (Table 2-3).

Both percent sequence variation and haplotype diversity were substantially lower in the Peace River for all disjunct species except *Papilio machaon*, whereas

continuously distributed species exhibited more similar levels of sequence divergence and haplotype diversity between regions, with the exception of *Erebia epipsodea*. *Plebejus saepiolus* exhibited the most sequence variation, with haplotypes differing by as much as 1% within each region.

Haplotype network diagrams provide more details about the haplotype diversity (Figures 2-5, 2-6). The two disjunct genera that were each represented by two species (*Oeneis* and *Satyrium*) displayed very different patterns. Haplotype frequency and distribution differed between the two *Oeneis* species (Figure 2-5). The 33 specimens of *Oeneis alberta* yielded 12 haplotypes, of which one was most common in both regions. The 40 *Oeneis uhleri* specimens yielded 23 haplotypes, none of which were shared between regions. One Peace River haplotype was overwhelmingly common, while none of the southern haplotypes were particularly common. In contrast, the number and distribution of haplotypes were similar in both *Satyrium* species (Figure 2-5). In each species a single haplotype was shared between regions and was very common in the Peace River region. The shared haplotypes were not particularly common in southern *S. titus* or *S. liparops*. Both species exhibited little genetic diversity in Peace River, with only two haplotypes each in the region.

For *Papilio machaon*, only *P.m. oregonius* exhibited a cluster of haplotypes that corresponded to a described subspecies (Figure 2-5). All four subspecies possessed haplotypes not found in the others, however one haplotype was shared between *P.m. dodi*, *P.m. pikei*, and *P.m. aliaska*, while a second was shared between *P.m. pikei* and *P.m. aliaska*. Four haplotypes (two *P.m. pikei* and two *P.m. aliaska*)

were unusually divergent, and only one could be included in the nested clade analysis (haplotype 3 in Figure 2-5).

The two continuously distributed Satyrinae exhibited different network and nested clade patterns from one another (Figure 2-6). *Coenonympha tullia* yielded 24 haplotypes, 12 from each region. One Peace River haplotype was too divergent to be included in the network. *Erebia epipsodea* yielded ten haplotypes, one of which was most common within both regions. Like the satyrine butterflies, the two continuously distributed Lycaenidae exhibited different haplotypic patterns (Figure 2-6). *Glaucopsyche lygdamus* yielded 8 haplotypes, one of which was shared and most common in both regions. *Plebejus saepiolus* was highly variable, yielding 25 haplotypes from 28 specimens. Only two haplotypes were found in two or more specimens, both in southern Alberta.

Papilio canadensis exhibited considerable haplotypic uniformity between regions (Fig 2-6). Of the nine observed haplotypes, three were shared between regions, and three were unique to each region. One shared haplotype was most common in southern Alberta, while a second was the most prevalent in the Peace River region. The shared haplotype common in southern Alberta was also found in specimens from Alaska and New York.

2.3.1 Statistical significance of genetic structure

For the disjunct species, AMOVA results (variance components and F statistics) demonstrated significant structuring between the Peace River and southern

populations for all discontinuously distributed species except *O. alberta* ($p<0.05$) (Table 2-4). Of the continuously distributed species, the Peace River and southern *C. tullia*, *E. epipsodea* and *P. canadensis* were significant at $\alpha=0.05$. At $\alpha=0.01$, only the four aforementioned disjunct species and *C. tullia* were significantly structured (Table 2-4). All ten species exhibited greater variance within populations than between populations (Table 2-4). Overall migration estimates (M-values) are generally higher for the continuously distributed species than for those with disjunct distributions in the Peace River region, with the most conspicuous exception being *O. alberta* (Table 2-4).

Exact tests of sample differentiation performed similarly to AMOVA (Table 2-5). Peace River and southern populations were significantly different for all the Peace River endemic species with the exception of *O. alberta* ($p<0.002$). Of the continuously distributed species, *C. tullia* and *E. epipsodea* and *G. lygdamus* also exhibited significant differences between populations.

Mantel Test correlation between genetic and geographic distance is shown in Table 2-5. Three of the five disjunct species showed a significant correlation, as did two of the continuously distributed species (*C. tullia* and *E. epipsodea*). Of the disjunct species, *O. alberta* and *P. machaon* were non-significant, though *P. machaon* was near significance when the divergent haplotypes were excluded ($p=0.072$).

The GeoDis component (Posada *et al.* 2000) of ANeCA (Panchal 2007, Panchal and Beaumont 2007) was used to determine whether any clades were significantly associated with particular geographic regions (nested contingency analysis). Significance was determined as the location of individuals within a clade

(D_c) relative to the mean location of all members of the nesting clade (D_n) (Templeton *et al.* 1995). Statistically significant clades are noted in Figures 2-5 and 2-6. Three species exhibited no significance (*O. alberta*, *P. canadensis*, and *P. saepiolus*), while the others were significant at least at the total cladogram.

The GeoDis inference key (Templeton *et al.* 1995, Templeton 2004) yielded various biogeographic inferences (see Table 2-6 for a summary). Allopatric fragmentation was inferred for part or all of three disjunct and one continuous species. The same numbers of species in each group showed contiguous range expansion. Several statistically significant clades could not be attributed to a particular biogeographic phenomenon (inconclusive outcome).

In Tables 2-3 through 2-5, the datasets are shown including and excluding the divergent *P. machaon pikei* and *C. tullia* haplotypes. Including the divergent haplotypes variously affected the values associated with AMOVA (variance components, Fst, M-value), exact tests of sample differentiation, and Mantel tests, however statistical significance remained unchanged (Table 2-4 and 2-5).

2.4 Discussion

2.4.1 Comparisons among species with similar distributions

Although species with similar geographic distributions generally had more similar patterns of genetic structuring, there were exceptions at several levels. AMOVA and exact tests of sample differentiation consistently separated the Peace River and

southern Alberta populations for all disjunct species except *O. alberta*, and *C. tullia* among the continuously distributed species.

The Mantel test results were similar to the AMOVA results, with some exceptions. Of the disjunct species, neither *O. alberta* or *P. machaon* showed significant association between genetic and geographic distances. With all four divergent haplotypes excluded, the *P. machaon* data approached significance, but may have been confounded by the small sample size (only 11 *P.m. pikei* remain once the divergent sequences are omitted). As with AMOVA, *C. tullia* and *E. epipsodea* demonstrated significant structuring however, neither species had a particularly high r^2 value (see Table 2-3), suggesting that there is substantial genetic variability that is not accounted for by geographic distance.

The haplotype networks were variable from species to species, both within and between disjunct (Figure 2-3) and continuous populations (Figure 2-4). Distances between haplotypes varied between all ten species, however, there were no consistent differences between continuous and disjunct species. *Plebejus saepiolus* was notable for the number of unique haplotypes, the sequence divergence between them, and the number of unresolved reticulations.

Nested clade phylogeographic analysis provides a nuanced interpretation of the phylogeography of each species. NCPA clearly demonstrates that there is substantial demographic structure in most of the disjunct species, although the actual inferences vary within and between species (Table 2-6). Inferences of allopatry are confined to *O. uhleri*, *S. titus*, *P. machaon* and *C. tullia*. Some other inferences seem biologically less plausible, considering the known range and dispersal capabilities of particular

species (e.g. contiguous range expansion in *S. liparops*). Furthermore, a number of clades show statistically significant associations between phylogenetic relatedness and geographic location, but their interpretation is phylogeographically inconclusive (Table 2-6).

NCPA inferences support a number of relatively recent demographic changes in Peace River butterflies, however their low levels of genetic divergence make it difficult to determine their respective causes. Templeton (2004) has argued that NCPA is conservative when assigning phylogeographic status, though there is considerable controversy concerning the believability of *a priori* and *a posteriori* assumptions being made in the application of NCPA (Knowles and Maddison 2002, but see Templeton 2004, Panchal and Beaumont 2007). Most of the phylogenetic inferences from Peace River populations are plausible given a prior knowledge of their distribution, however the less credible ones (i.e. contiguous range expansion) warrant further investigation. Examination of additional rapidly evolving genes may help resolve this problem.

Populations of *Oeneis alberta* were not significantly structured in any of the analyses. It is possible that the Peace River populations simply have not diverged from the widespread ancestral haplotype. However, this does not explain why *O. uhleri*, a congener with a very similar natural history, exhibits greater differentiation from the southern populations. It is unlikely that the Peace River populations of *O. alberta* represent a recent introduction (i.e. through agricultural movement), as there are several haplotypes present. Moreover, coloration differences have been noted in the Peace River populations of *O. alberta* and *O. uhleri* (Case and Bird 1977, Kondla

et al. 1994). In both species, the Peace River haplotypes are highly similar (Figure 2-3), suggesting that haplotype diversity at the time of isolation did not play a strong role in the current disparity. Interestingly, removing 20 bases from the end of the sequence removes a single variable base, which in turn yields a significant F statistic and NCPA inference (Bromilow 2007 Chapter 3). This suggests that there is some significant phylogeographic structure in the species that may have been more recently obscured by conflict in the phylogenetic signal.

The highly divergent haplotypes seen in *P. machaon* and *C. tullia* occur too consistently to be considered contamination or misidentification. The highly divergent haplotypes of *P. machaon*, coupled with the greater uniformity seen in all other haplotypes, suggests that the North American populations may have undergone a selective mitochondrial sweep with surviving refugial pockets. This is reinforced by the placement of the French *Papilio machaon gorganus* as closely related to North American populations (see Figure 2-4). This is plausible given the frequency of hybridization within this species group (Sperling 1987, Sperling and Harrison 1994). If the divergent haplotypes do in fact represent an incomplete mitochondrial selective sweep, it would indicate that mtDNA analyses might not uncover otherwise divergent populations in *Papilio*, as has been the case in some Lepidoptera (Nice *et al.* 2002, Gompert *et al.* 2006). Regardless of the reason, the haplotypic disparity within and between subspecies of the *Papilio machaon* group suggests that single-gene based methods of species identification (i.e. DNA barcoding, Hebert *et al.* 2003) should be approached cautiously. Further work is necessary, and requires sampling the entire species complex more thoroughly throughout its range.

The divergent haplotype lineage of *C. tullia* may represent a cryptic species, or some sort of highly aberrant mitochondrial type. These individuals were caught alongside genetically 'normal' individuals. Initial morphological examination revealed no clear difference between the two genetic types. The haplotype is not genetically close to any North American populations of *Coenonympha* that I have examined thus far (Figure 2-5). Further work is underway using detailed morphological and genetic analyses to characterize this population and this unusual species group.

Coenonympha tullia exhibited several other unexpected features. Even when the divergent haplotypes are excluded, the strong phylogenetic structuring of the remaining haplotypes (see Figures 2-4 and 2-6) suggests that *C. tullia* is not in fact continuously distributed, but rather may be cryptically disjunct. However, genetic diversity was approximately equal between the two regions, suggesting that this species has not experienced the reduction in genetic diversity seen in the Peace River populations of other disjunct species. Other authors (Guppy and Shephard 2001 for example) have suggested considerable structuring of this species group based on morphological variability across the continent, even in the Peace River region (Kondla *et al.* 1994). Geographic structuring is further reinforced by the placement of our *C. nipisiquit* and California *C. tullia* specimens (see Figure 2-4).

The extreme genetic variability observed in *Plebejus saepiolus* showed no clear haplotypic patterns, and no apparent population structure. Interestingly some other Lepidoptera have been found to exhibit the opposite trend, notably a lack of mtDNA differentiation between morphologically distinct populations (for example

Lycaeides blues [Gompert *et al.* 2006], and *Grammia* tiger moths [Schmidt PhD 2007]). *Plebejus saepiolus* may represent a particularly rapidly evolving mitochondrial lineage, though there is no obvious other support for this interpretation. Other genes, including those with lower mutation rates, would need to be examined in order to determine whether or not this variability is representative of the rest of the genome of the species.

While there were observable trends within disjunct versus continuous species, divergence patterns were not very consistent even for congeneric pairs of species that have similar distributions. For example, the two *Satyrium* species exhibited similar genetic trends while the two *Oeneis* species were dissimilar. Analysis of *O. alberta* alone would suggest that the Peace River butterflies have undergone little or no genetic diversification, while *O. uhleri* would suggest that they have undergone substantial genetic diversification. Examination of only the hairstreak butterflies would suggest that the Peace River populations have undergone a major bottleneck with little subsequent genetic diversification. The *Papilio* dataset suggests a more complex scenario, possibly caused by historical divergence following an incomplete barrier to gene flow. It is important to note that these interpretations may be valid for each given species, but cannot be used to accurately characterize other species within a broader group (in this case, all grassland butterflies). Thus conservation decisions for a region should not be based on the degree and pattern of genetic divergence of a single disjunct species.

2.4.2 Comparisons between species with different distributions

Several general trends are evident in comparisons among groups of species with different geographic distributions. Species with disjunct ranges generally had fewer haplotypes, lower haplotype diversity, and lower mean sequence divergence than did those with continuous ranges. In addition, more of the disjunct species had significant genetic structuring between regions, as determined by AMOVA, exact tests and nested clade analysis, as well as Mantel tests of association between genetic and geographic distances.

However, divergence in the disjunct species can be more accurately characterized in the context of the continuously distributed populations. There were consistent differences between the disjunct and continuous species, although these too varied in degree between taxa. The generally low genetic diversity (haplotype diversity, % sequence variation) in the disjunct Peace River populations suggests that they have undergone processes such as rapid range expansion, founder effects, or population bottlenecks. This, combined with the significant population structuring and relatively consistent clade grouping of the disjunct species (with the exception of *Oeneis alberta*), also suggests that Peace River populations are isolated. However, some divergence across geographic distance was observed in all continuously distributed species (e.g. regionally isolated haplotypes), indicating that no species are completely panmictic. Isolated haplotypes in *E. epipsodea*, *G. lygdamus* and *P. canadensis* further indicate that these continuously distributed species exhibit restricted gene flow between regions. Estimates of the number of migrants between the regional sample populations per generation (M-values) were on average higher for the

continuously distributed species (mean 4.04 excluding the outlying *P. saepiolus* value, Table 2-4); however the disjunct species still displayed an average value of 1.30 (excluding the outlying *O. alberta* value, Table 2-4). Thus not all genetic divergence between regions in species with disjunct distributions can be attributed to geographic isolation; a substantial proportion may in fact be caused by isolation across geographic distance.

Other factors suggest that Peace River grassland butterflies do show genetic divergence and structuring due to disjunction alone. Collinge (2000) has shown that butterflies with disjunct distributions tend to exhibit lower genetic diversity and overall variability than those with large continuous populations. In my study, haplotype and genetic variation were similar between regions in the continuously distributed species, whereas these parameters showed more divergence between the regions of disjunct species. Moreover, population structuring was not as consistently significant in the continuous species. At α=0.05, *Erebia epipsodea*, *Papilio canadensis* and four disjunct species exhibited significant population structuring (Table 2-2), while at a stricter α value (0.01), significance was only seen in the disjunct species. This is further reinforced by lower relative significance seen in the exact tests of sample differentiation in *E. epipsodea* and *G. lygdamus*.

It is possible that regionally unique 'Peace River haplotypes' seen in both disjunct and continuously distributed species are artefacts of sampling (i.e. these haplotypes exist in southern Alberta but were not found). However, three of the five disjunct species plus *C. tullia* exhibited Peace River haplotypes that were represented by multiple specimens. Of the continuous species, this was only true for *E. epipsodea*.

Attenuated gene flow caused by patchy or scattered habitat or even simple diffusion dynamics with low dispersal distances may explain the isolated haplotypes seen in the continuously distributed species (Neve *et al.* 1996, Keyghobadi *et al.* 1999).

2.4.3 Conservation implications

Accurate characterization of geographically isolated populations is of paramount importance to future conservation efforts (Haig *et al.* 2007). Multi-species comparisons can make conservation decisions much more robust, as consistent phylogeographic patterns will help elucidate the nature and origin of isolated populations (Soltis *et al.* 1997, Avise 1998, Knowles and Maddison 2002, Soltis *et al.* 2006). This approach is especially important under conditions of recent isolation and/or low divergence. Comparative studies that have shown relatively consistent large-scale phylogeographic or genetic trends (i.e. between relatively divergent populations), while smaller scale (i.e. more recent or local) changes can vary greatly from group to group (Satoh *et al.* 2004, DeChaine and Martin 2005, Painter *et al.* 2007). The recently isolated Peace River grassland butterflies suggest that conservation priorities could easily be mislaid if inferences rely on single species.

Examination of the population structure of Peace River grassland butterflies shows that recently diverged disjunct populations can appear similar to those isolated solely by geographic distance. The recent history of climate change and glaciation in North America and Eurasia has lead to the isolation of many species in similar disjunct habitats (Soltis *et al.* 1997, Taberlet *et al.* 1998), thus highlighting the

importance of this problem. By comparing geographically isolated species to continuously distributed ones, I can effectively control for this, thereby strengthening conservation decisions. This then provides a method for distinguishing biotic assemblages whose divergence is significantly explained by geographic isolation.

Gauthier and Wiken (2003) estimate that only 25-30% of Canada's native grasslands are intact, and only 3.5% of those are protected. Disjunct regions and fragments within a larger habitat are particularly susceptible to disturbance and climate change, and are therefore important from a conservation perspective (McLaughlin *et al.* 2002). Grassland butterflies are especially important to the conservation of these regions for two reasons. First, specialist butterflies within a region (i.e. those with high habitat or host-plant specificity) are extremely important for monitoring, as they are most likely to decline in the face of moderate or severe disturbance (Hogsden and Hutchison 2004). Second, the complex nature of genetic variation at range edges further emphasizes the need to study multiple broadly related species (Hampe and Petit 2005, Arnaud-Haond *et al.* 2006, Bridle and Vines 2006). The Peace River grassland butterflies thus provide an important faunal group for informing management decisions about the disappearing remnants of the Peace River grasslands in western Canada.

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Table 2-1. Collection localities with geographic coordinates.

Locality	Name*	Latitude	Longitude
1	Monument to 12' Davis, Peace River	56.234	-117.272
2	Kleskun Hill Park	55.258	-118.504
3	Road to Green Island, south of Fairview	55.917	-118.442
4	Fairview Ski Hill	56.081	-118.399
5	Misery Mountain, Peace River	56.214	-117.296
6	Lac Cardinal	56.253	-117.823
7	Dunvegan	55.926	-118.596
8	East of Woking	55.608	-118.769
9	Road to Judah, south of Peace River	56.202	-117.286
10	South of Cecil Road, BC	56.266	-120.627
11	Taylor, BC	56.177	-120.665
12	South of Bear Canyon	56.180	-119.745
13	Peace River townsite	56.234	-117.290
14	Foster's Point, south of Peace River	56.118	-117.408
15	Peace Point, Peace River	56.234	-117.272
16	South of Scotswood	56.091	-118.682
17	Blakley's Site, southwest of Peace River	56.087	-117.575
18	Tangent Park	56.092	-117.542
19	Wildland Park	56.119	-117.407
20	Bear Canyon	56.211	-119.774
21	High Level	58.519	-117.120
22	Lot 27, Peace River	56.147	-117.464
23	Shaftesbury Trail, southeast of Peace River	56.161	-117.420
24	Highland Park	56.109	-118.900
25	East of Ksituan	55.730	-118.476
26	South of Cleardale	56.229	-119.519
27	Fort St. John, BC	56.206	-120.783
28	North of Waiparous	51.287	-114.840
29	Moose Mountain Road, Bragg Creek area	50.954	-114.578
30	Fish Butte, south of Bragg Creek	50.923	-114.564
31	Beaver Creek Recreation Area	49.806	-113.934
32	Drumheller	51.443	-112.709
33	Helen Schuler Coulee Center, Lethbridge	49.699	-112.861
34	North Lethbridge	49.704	-112.854
35	Popson Park, Lethbridge	49.644	-112.864
36	Southeast Lethbridge	49.680	-112.844
37	Cottonwood Park, Lethbridge	49.635	-112.892
38	Akasu Hill east of Vegreville	53.480	-111.914
39	Itaska, Pigeon Lake	53.065	-114.075
40	Pigeon Lake Provincial Park	53.024	-114.130
41	Mackinnon Ravine, Edmonton	53.539	-113.560

42	Ada Boulevard, Edmonton	53.565	-113.415
43	Fulton Ravine, Edmonton	53.545	-113.435
44	Strathcona Science Park, Edmonton	53.562	-113.377
45	Airdrie	51.302	-114.007
46	Athabasca	54.715	-113.293
47	Hillcrest	49.572	-114.371
48	Veinerville, near Medicine Hat	49.962	-110.603
49	South of Manyberries	49.191	-110.732
50	Carbon	51.517	-113.280
51	Dinosaur Provincial Park	50.756	-111.529
52	Chinook Butterfly Circle	51.301	-110.950
53	Tollman Bridge	51.830	-110.000
54	South of Chauvin	52.583	-110.142
55	Jenner Bridge	50.836	-111.170
56	Elnora	52.001	-113.205
57	Cadomin Trail	52.998	-117.334
58	Wainwright Dunes	52.593	-110.594
59	Southeast of Edgerton	52.721	-110.434
60	Pakowki Lake	49.398	-110.875
61	Hilda Sand Dunes	50.455	-110.078
62	Waterton Lakes National Park	49.099	-113.905
63	Good Spirit Lake, SK	51.561	-102.711
64	North Battleford, SK	52.767	-108.283
65	Crooked Lake, SK	50.610	-102.762
66	McCone County, MT	47.352	-105.592
67	West of Glenboro, MB	49.600	-99.336
68	Wishram, WA	45.657	-120.965
69	Thompson Culture, WA	47.390	-120.363
70	Klickitat, OR	45.592	-121.153
71	Pink Mountain, BC	57.042	-122.523
72	Eagle Summit, AK	65.293	-145.149
73	Fairbanks, AK	64.826	-147.928
74	Richford, NY	42.359	-76.207

* Localities are in Alberta unless otherwise indicated by a 2-letter acronym for the province or state.

Table 2-2. Primers used for PCR amplification.

Primer Name	Sequence	Reference
TY-J-1460a 'K698'	TACAATTTATCGCCTAAACTTCAGCC	Sperling et al. 1994
C1-J-1495 'Lyn'	ACAAATCATAAGGATATTGGAAC	New
C1-N-1840 'Brent'	GGGTAAACTGTTCATCCTGTTCC	New
C1-N-1840a 'K699'	AGGAGGATAAACAGTTCAC/TCC	Sperling et al. 1995
C1-N-1840b 'K791'	TGGGGGGTATACTGTTCAT/ACC	New
C1-J-1751e 'RonV'	GGAGCTCCAGATATAGCTTTCCC	Du et al. 2005
C1-J-2183a 'Jerry'	CAACATTTATTTTGATTTTTTGG	Simon et al. 1994
C1-N-2329 'K525'	ACTGTAAATATATGATGAGCTCA	Simon et al. 1994
C1-J-2531 'Ellen'	TTTACTGTAGGAGGATTAACWGG	Du et al. 2005
C1-N-2578a 'K741'	TGGAAATGTGCAACTACATAATA	Caterino and Sperling 1999
TL2-N-3013 'Pat'	TCCATTACATATAATCTGCCATATTAG	Sperling et al. 1996

Table 2-3. Haplotype and sequence heterogeneity.

Species	Total haplotypes		Haplotype diversity			Mean % sequence divergence		
	PR	SA	PR		SA	PR		SA
O. uhleri	7	16	0.605	<	0.925	0.055±0.046	<	0.285±0.166
O. alberta	5	8	0.477	<	0.711	0.040±0.038	<	0.102±0.073
S. titus	2	8	0.106	<	0.833	0.008±0.015	<	0.178±0.116
S. liparops	2	5	0.142	<	0.720	0.021±0.026	<	0.109±0.075
P. machaon di	5	6	0.741	>	0.725	0.666±0.360	>	0.171±0.110
P. machaon de	3	6	0.595	<	0.725	0.107±0.078	<	0.171±0.110
C. tullia di	12	12	0.814	<	0.848	1.288±0.658	>	0.202±0.124
C. tullia de	11	12	0.787	<	0.848	0.228±0.138	>	0.202±0.124
E. epipsodea	3	8	0.227	<	0.749	0.017±0.023	<	0.139±0.092
G. lygdamus	3	6	0.540	>	0.507	0.044±0.041	<	0.075±0.058
P. saepiolus	14	11	0.929	>	0.918	0.907±0.495	>	0.612±0.337
P. canadensis	6	6	0.761	>	0.554	0.083±0.063	>	0.063±0.051

Numbers after ± symbol indicate standard deviations. *Papilio machaon* covers Alberta haplotypes only. *Coenonympha tullia* and *Papilio machaon* datasets are shown with divergent haplotypes included (di) and excluded (de).

Table 2-4. Sources of genetic variation (AMOVA) between (B) and within (W) Peace River and southern populations and F statistics with significance indicating population differentiation.

	Variance Component		% of variation				
Species	B	W	B	W	Fst	Fst p-value	M-value
O. uhleri	0.097	0.403	19.47	80.53	0.195	<0.0001*	2.064
O. alberta	0.007	0.314	2.28	97.72	0.023	0.161±0.011	21.239
S. titus	0.133	0.212	38.45	61.55	0.384	<0.0001*	0.802
S. liparops	0.171	0.259	39.71	60.29	0.397	<0.0001*	0.759
P. machaon di	0.102	0.392	20.64	79.36	0.206	<0.0001*	1.927
P. machaon de	0.328	1.021	24.62	75.68	0.243	0.00033±0.0003*	1.558
C. tullia di	2.044	5.977	25.48	74.52	0.255	0.00198±0.0009*	1.461
C. tullia de	0.069	0.431	13.74	86.26	0.137	<0.0001*	3.150
E. epipsodea	0.048	0.260	15.61	84.39	0.156	0.016 ±0.004*	2.705
G. lygdamus	0.027	0.279	8.96	91.04	0.090	0.080±0.010	5.056
P. saepiolus	0.003	0.497	0.55	99.45	0.005	0.500±0.000	99.500
P. canadensis	0.033	0.345	8.68	91.32	0.087	0.0503±0.0036**	5.247

* Significant at _ = 0.05

** Standard deviation includes significance at _ = 0.05

Numbers after ± symbol indicate standard deviations. *Papilio machaon* covers Alberta haplotypes only. *Coenonympha tullia* and *Papilio machaon* datasets are shown with divergent haplotypes included (di) and excluded (de).

Table 2-5. Exact test of sample differentiation based on haplotype frequencies and Mantel test, comparing Peace River and Southern Alberta populations.

Species	Exact Test	Mantel Test	
	p-value	R^2	p-value
O. uhleri	<0.0001*	0.230	0.001*
O. alberta	0.0648 ±0.0086	0.0002	0.479
S. titus	0.0015±0.0017*	0.290	0.001*
S. liparops	<0.0001*	0.313	0.001*
P. machaon di	<0.0001*	0.042	0.266
P. machaon de	<0.0001*	0.060	0.072
C. tullia di	<0.0001*	0.058	0.001*
C. tullia de	<0.0001*	0.089	0.001*
E. epipsodea	0.0065±0.0022*	0.111	0.026*
G. lygdamus	0.0116±0.0041*	0.014	0.815
P. saepiolus	0.4665±0.0320	0.004	0.640
P. canadensis	0.0961±0.0072	0.018	0.805

*Significant at $\alpha = 0.05$

Papilio machaon includes Alberta haplotypes only. *Coenonympha tullia* and *Papilio machaon* datasets are shown with divergent haplotypes included (di) and excluded (de). R^2 values are shown for log-transformed data.

Table 2-6. Demographic inferences from automated nested clade analysis (ANeCA)

Clade	Inference chain	Conclusion
O. uhleri		
1-6	1-2-11-17-NO	A
2-2	1-2-3-4-NO	B
2-4	1-2-IO	A
3-1	1-2-11-12-NO	C
TC	1-19-NO	D
O. alberta		
none	-	-
S. titus		
2-1	1-19-NO	D
TC	1-19-NO	D
S. liparops		
TC	1-2-11-12	C
P. machaon		
2-1	1-19-NO	D
3-2	1-19-20-2-3-4-NO	B
TC	1-2-11-12-NO	C
C. tullia		
1-6	1-19-20-2-11-12-NO	C
3-1	1-19-NO	D
3-2	1-2-11-12-13-14	E
TC	1-2-IO	A
E. epipsodea		
TC	1-2-IO	A
G. lygdamus		
TC	1-2-11-17-NO	A
P. saepiolus		
none	-	-
P. canadensis		
none	-	-

Only statistically significant clades are shown. All clades correspond to those marked with an asterisk in Figures 2-3 (disjunct) and 2-4 (continuous). *Coenonympha tullia* excludes highly divergent haplotype, *Papilio machaon* includes four subspecies but only one divergent haplotype from *P. machaon pikei* (see Materials and Methods for explanation).

A= Inconclusive

B= Restricted gene flow with isolation by distance

C= Contiguous range expansion

D= Allopatric fragmentation

E= Long-distance colonization and/or past fragmentation

Figure 2-1. Collecting localities for disjunct grassland butterflies in Alberta, and adjacent regions. Bold numbers indicate localities. Numbers following a colon indicate haplotypes found at a given locality, with the number of specimens given in brackets (except where n=1). Locality numbers correspond to Table 2-1. Arrows indicate collecting localities outside of the mapped area, with a two-letter province or state abbreviation. The box containing 'n=x' or subspecific names indicates the total number of specimens per region (Peace River or southern grasslands). The dashed diagonal line across Alberta indicates the midline between the Peace River region and southern Alberta grasslands.

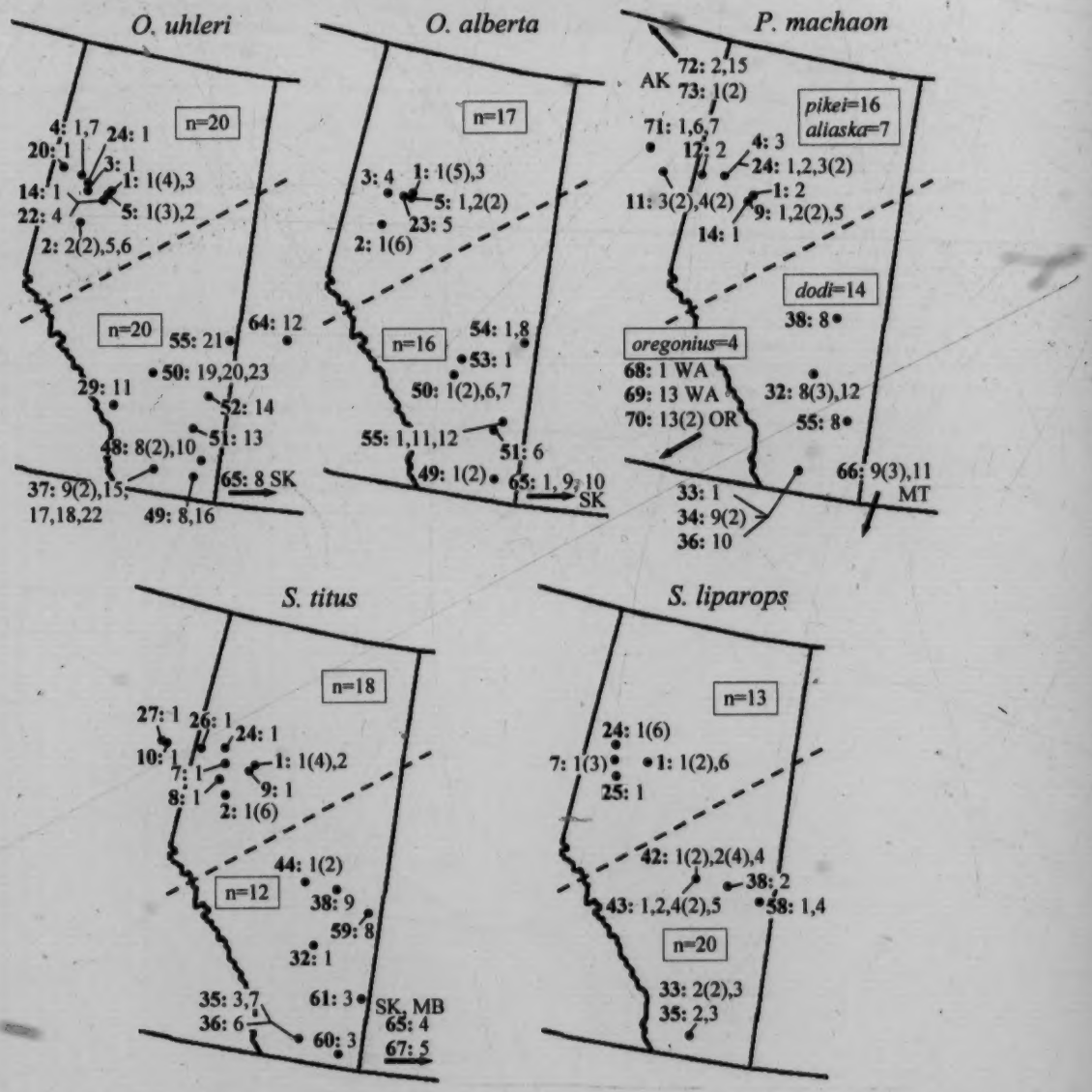


Figure 2-2. Collecting localities for continuously distributed butterflies in Alberta and adjacent regions. Format and symbols as on Figure 2-1.

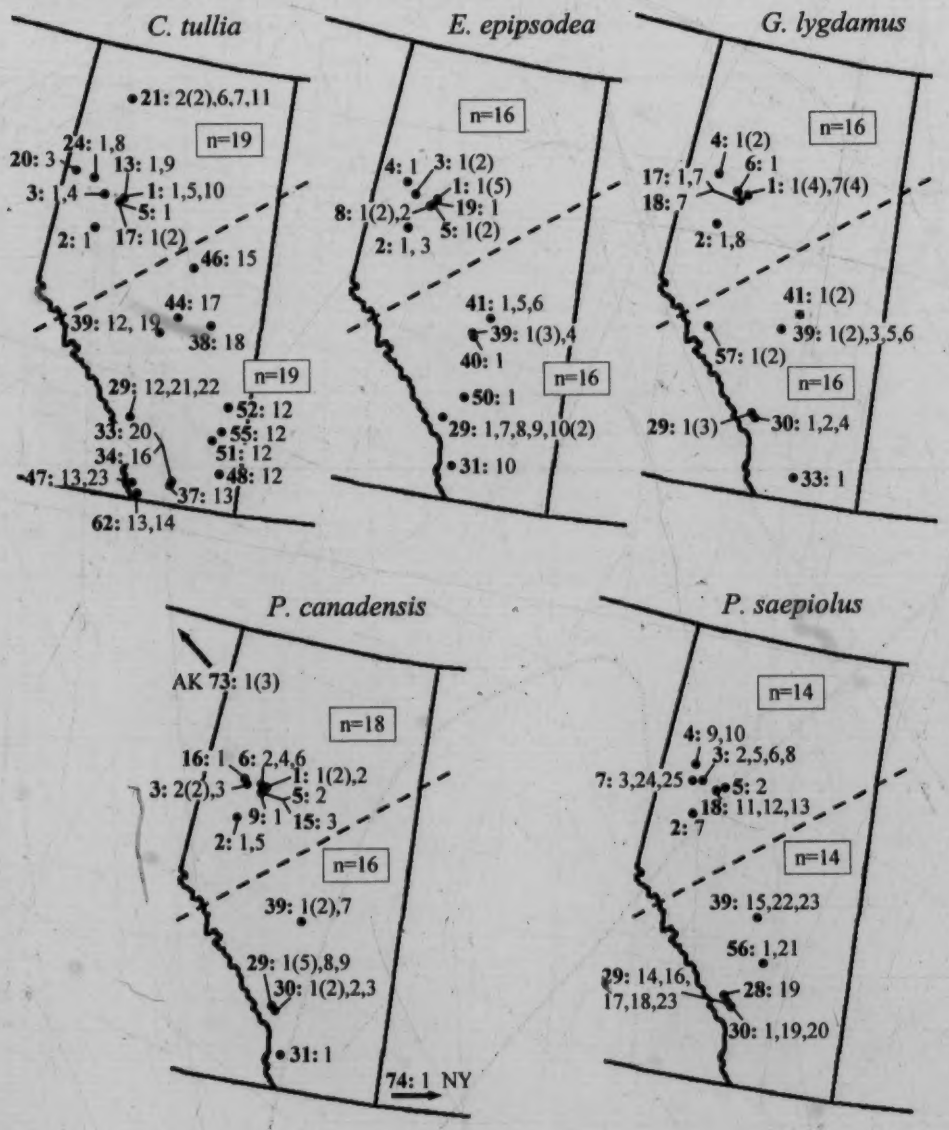


Figure 2-3. *Papilio machaon* phylogram derived from heuristic parsimony search showing the strict consensus of 1000 replicates. Haplotypes correspond to those in Fig 2-5. The subspecies is given after each haplotype, followed by a number in parentheses indicating the number of specimens with the given haplotype.

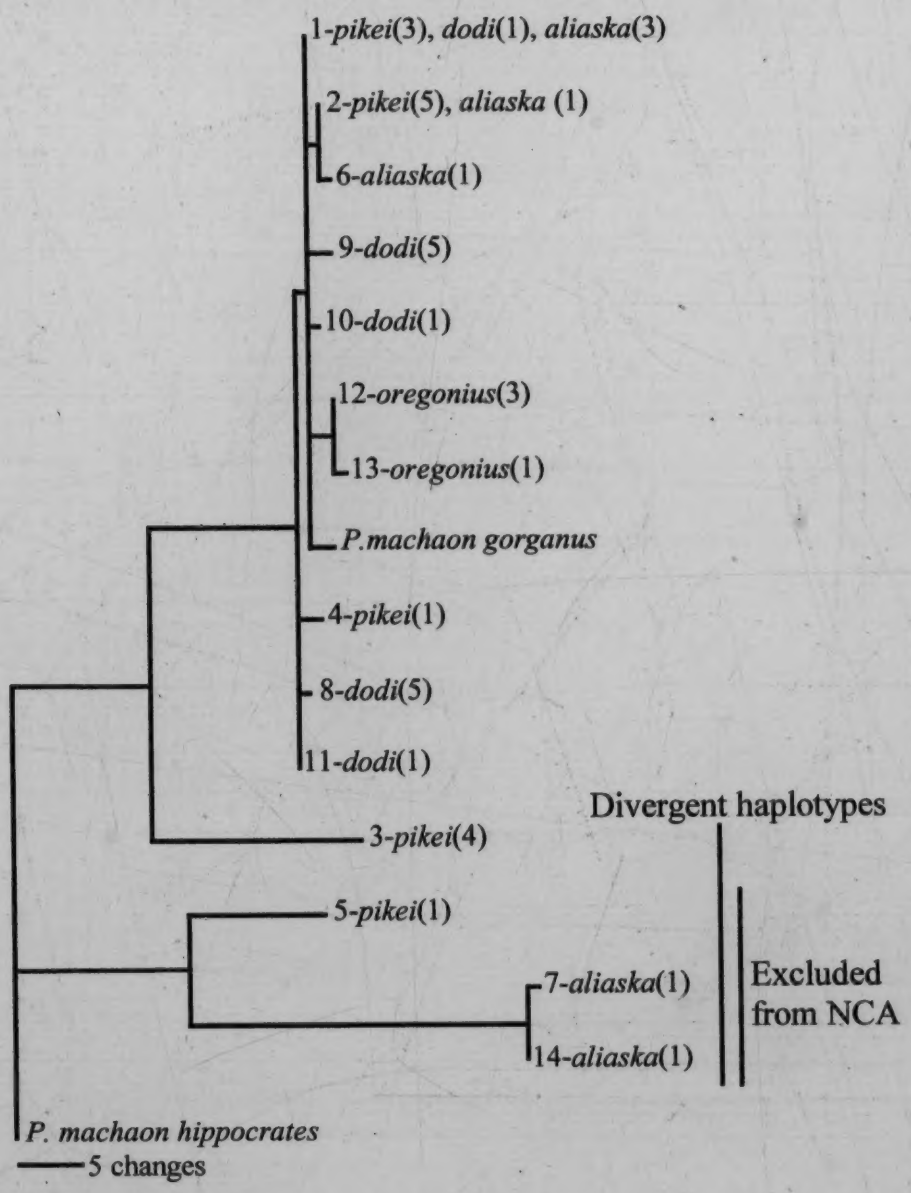


Figure 2-4. *Coenonympha tullia* phylogram derived from heuristic parsimony search showing the strict consensus of 1000 replicates. Haplotypes correspond to those in Fig 2-6, followed by a number in paretheses indicating the number of specimens with the given haplotype. Two Peace River groupings are indicated by vertical lines and 'PR1' and 'PR2.' Two southern Alberta clades are indicated similarly, with SA in place of PR. European *Coenonympha pamphilus* is used here as the outgroup.

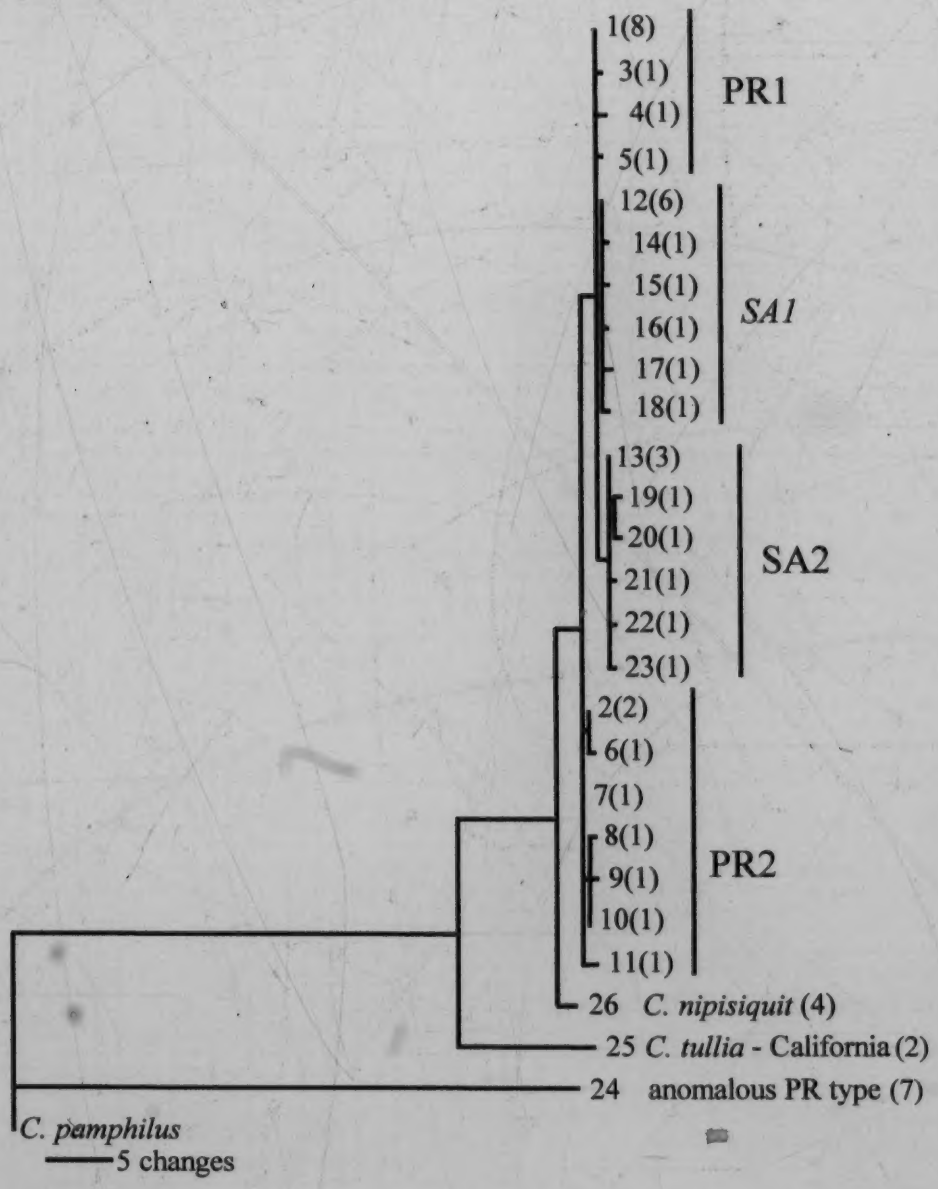


Figure 2-5
Haplotype network and clade nesting for five disjunct grassland species examined in this study. Numbers inside circles represent haplotypes. The number of individuals with a given haplotype is indicated by n=x, except where n=1. Small, empty circles indicate theoretical intermediate haplotypes connecting observed haplotypes. Light grey fill indicates Peace River specimens/haplotypes. White fill indicates southern Alberta specimens/haplotypes. In *Papilio machaon*, light grey fill indicates *P.m. pikei*, white is *P.m. dodi*, dark grey is all *P.m. aliaska*, while black is *P.m. oregonius*. Partitioned circles represent haplotypes shared between regions or subspecies. Dotted boxes with round corners, solid boxes, dashed boxes and dotted boxes with square corners indicate one-, two-, three-, and four-step clades respectively. Nested clades are indicated by two numbers separated by a dash; the first number indicates the level of nesting, while the second identifies the clade. TC indicates the total cladogram. Cladogram roots are indicated by an 'R' in a box. Within the *machaon* tree, clades 1-10 to 1-13 are indicated by the letters a-d respectively, while clades 2-5 and 2-6 are indicated by the letters E and F. Lines between haplotypes indicate single mutational steps. Line length is arbitrary. Statistically significant clades are indicated by an asterisk (*).

Figure 2-5

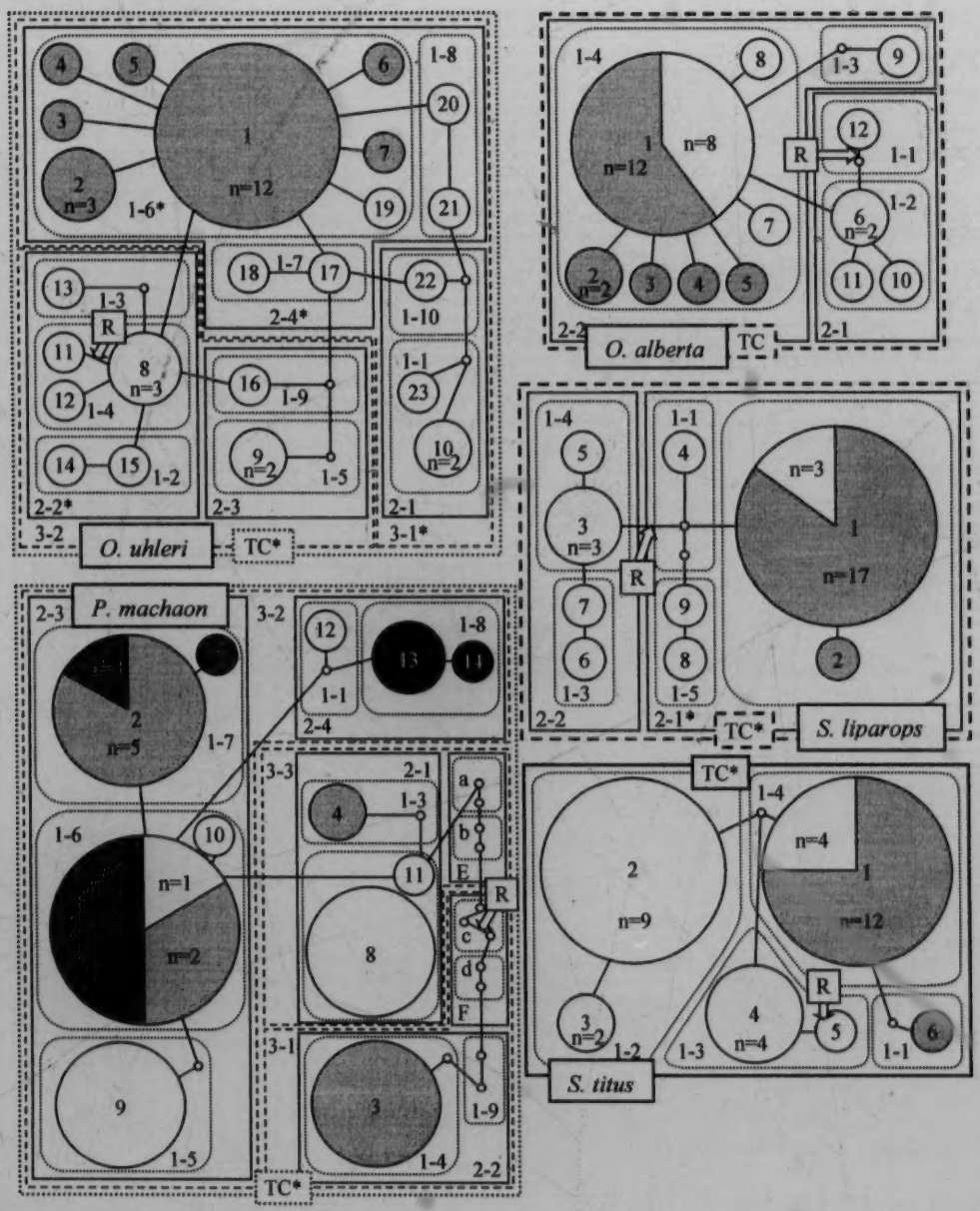
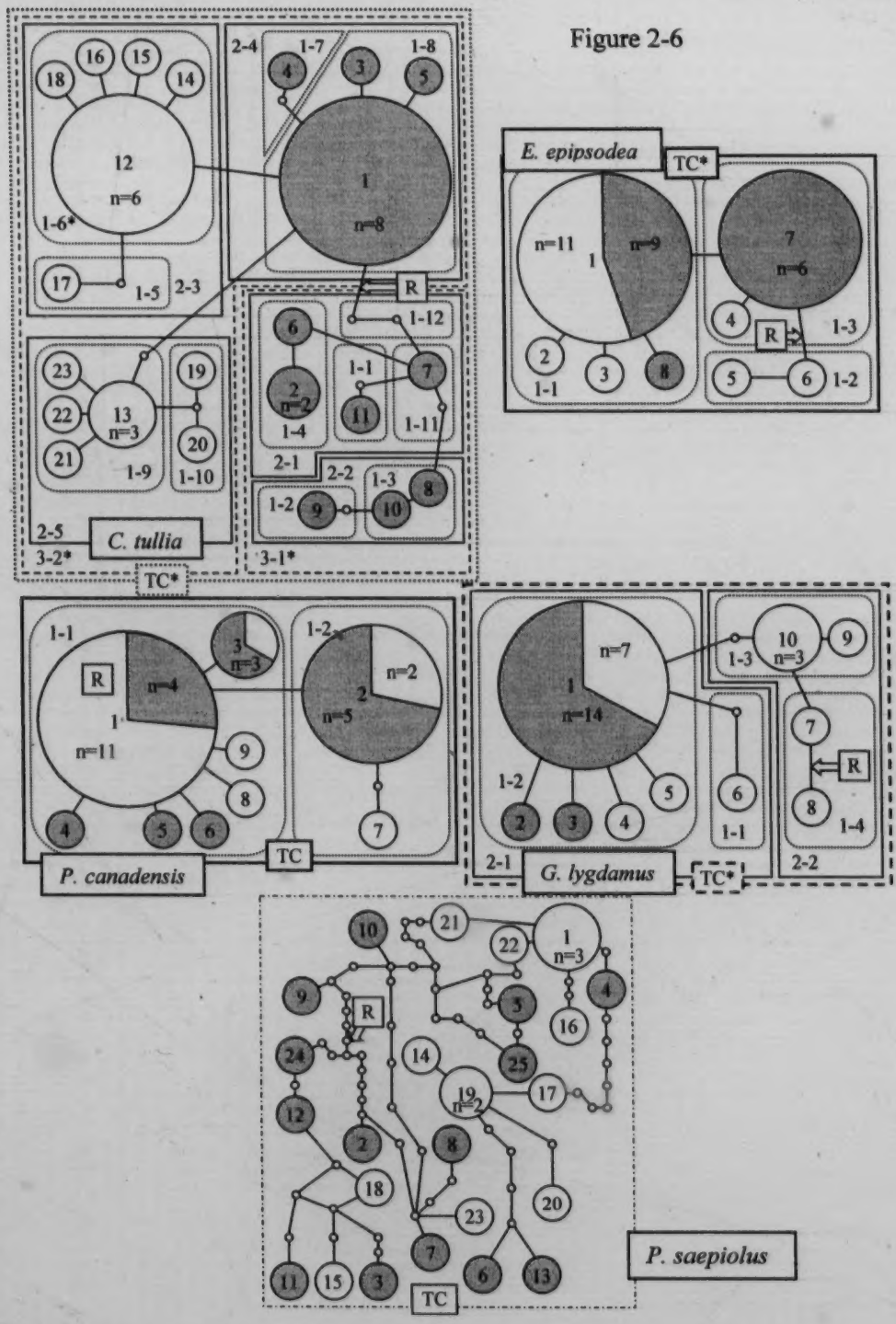

O. uhleri
TC*
O. alberta
TC
S. liparops
TC*
P. machaon
TC*
S. titus
TC*
n=12
n=8
n=17
n=12
n=9
n=5
n=3
n=2
n=4
n=2
n=3
n=2
n=1
n=2
n=4
1-6*
2-2*
2-4*
1-10
2-3
2-1
3-2
3-1*
1-4
1-3
1-1
1-2
2-2
2-1*
1-5
1-7
2-4
1-8
3-3
3-1
1-9
1-4
2-2
R

Figure 2-6
Haplotype network and clade nesting of the five continuously distributed species examined in this study. Format and symbols as Fig. 2-5. The *P. saepiolus* diagram does not include any nesting boxes due to space constraints; the overall nesting arrangement consisted of 160 named clades, none of which were significant.

Figure 2-6


E. epipsodea
TC*
n=11
n=9
n=6
R
1-1
1-2
1-3
C. tullia
TC*
n=3
1-9
1-10
2-5
3-2*
3-1*
1-4
1-11
2-1
2-2
1-12
n=2
n=4
n=5
P. canadensis
TC
n=7
n=14
n=3
1-4
2-1
2-2
G. lygdamus
TC*
n=3
P. saepiolus
TC

Chapter 3: USE OF BARCODE-LENGTH OR SHORTER MITOCHONDRIAL DNA SEQUENCES LEADS TO CAPRICIOUS EVOLUTIONARY AND CONSERVATION INTERPRETATIONS

3.1 Introduction

Mitochondrial DNA has long been used to identify and assess populations and species, including those of conservation concern (Moritz 1994). MtDNA is easy to use as there are multiple copies per cell, and there are robust primers for many taxa. The molecule provides excellent information for reconstructing evolutionary paths – because recombination is very limited, it has a rapid evolutionary rate, and its maternal inheritance makes it effectively haploid (Avise *et al.* 1987, Avise 1991). However the mtDNA molecule is 15 kb long in most animals, which is impractical to sequence fully in more than a few specimens (but see Ballard and Rand 2005). Moreover, it is not immediately obvious which particular region or length of mtDNA provides optimal phylogeographic or phylogenetic information (Caterino *et al.* 2000, Erpenbeck *et al.* 2005, Roe and Sperling 2007a).

The 1.5 kb cytochrome *c* oxidase subunit I (COI) gene of mtDNA has been proposed as an ideal region for characterizing relatively recent divergence between insect populations because of its divergence rates, lack of indels and prominent past use in research (Caterino *et al.* 2000). Hebert *et al.* (2003a, b) have taken this a step further, recommending that a 648 bp fragment of the 5' end of COI be used in the identification and delimitation of all animal life (i.e. DNA barcoding). However,

there is ongoing debate over the general efficacy and accuracy of DNA barcoding (Sperling 2003, Ebach and Holdredge 2005, Hebert and Gregory 2005, Meyer and Paulay 2005, Will and Rubinoff 2004, Rubinoff 2006a, b).

Gene-tree monophyly has been important from both conservation (Moritz 1994) and phylogeographic (Avise 2000) standpoints. However, the stochastic processes that lead to this state may take a great deal of time and monophyly itself may have limited evolutionary significance (Neigel and Avise 1986, Hudson and Turelli 2003). However, isolated populations that do not exhibit reciprocal monophyly can be characterized by other methods, including variation in haplotype diversity and genetic diversity, nested clade inferences, and population differentiation measures (e.g. F statistics) (Excoffier *et al.* 1992, Templeton *et al.* 1995, Knowles 2004). Omland *et al.* (2006) have proposed a standard nomenclature for the polyphyletic stages of intermediate divergence (i.e. the stages leading up to gene-tree monophyly). In their system, there are three precursor stages to gene-tree monophyly. The first is rampant polyphyly, which includes 1a) haplotype panmixia and 1b) differences in haplotype frequency between populations. The second state is true intermediate polyphyly, which includes 2a) neotypy, in which only ancestral haplotypes are shared between populations, with new haplotypes having evolved regionally, 2b) allotypy, in which no haplotypes are shared between regions but there is no sorting of lineages, and 2c) allophyly, in which no haplotypes are shared and populations begin to form monophyletic subclades. Finally, species can exhibit 3) simple paraphyly, in which one population is monophyletically nested within another. This system is potentially a very useful contributor to conservation decisions, as

isolated habitats may be home to recently diverged taxa that are difficult to characterize (Omland *et al.* 2006). However, the application of this method of characterization of non-monophyletic patterns needs to be examined in a range of species and sequence lengths beyond those used by Omland *et al.* (2006).

I examined five disjunct butterfly species from the Peace River region of northern Alberta and British Columbia, Canada. These butterflies were isolated by climate change some 6000-9000 years ago (Strong and Hills 2003). These five species were compared to five species from the same families, which had continuous distributions across Alberta (i.e. found from the Peace River region continuously south to the U.S. border). These continuous species act as a control for simple isolation by distance rather than disjunction (Bromilow Chapter 2). I extracted and sequenced a 1420 bp region of mtDNA, spanning most of the COI gene in multiple specimens for each species. These sequences were then divided into halves (710 bp), thirds (473/474 bp), and quarters (355 bp). These shorter segments of COI were used to examine the length of sequence that is effective for accurately characterizing intermediate divergence and genetic diversity. By using multiple species (including both disjunct and continuously distributed species), I was able to examine the consistency of these trends between species and conservation units.

3.2 Materials and methods

3.2.1 Taxonomic sampling and molecular methods

I examined mtDNA in 347 specimens from ten butterfly species (Appendix 1). Of these, seven individuals had been used in previous studies (Caterino and Sperling 1999, Caterino *et al.* 2001, Zakharov *et al.* 2004, Pena *et al.* 2006). The ten species were chosen on the basis of their distribution within the Peace River grasslands of northern Alberta and British Columbia, Canada. Five species have disjunct distributions restricted to the Peace River region and grasslands further south, with at least a 300 km gap between these areas, while the remaining five are more continuously distributed throughout the province of Alberta. The five disjunct grassland-associated species are *Oeneis alberta* Elwes and *O. uhleri* (Reakirt) (Nymphalidae, Satyrinae), *Satyrium titus* (Fabricius) and *S. liparops* (Le Conte) (Lycaenidae, Theclinae), and *Papilio machaon* Linnaeus (Papilionidae, Papilioninae). The five continuously distributed species are *Coenonympha tullia* (Müller) and *Erebia epipsodea* Butler (Nymphalidae, Satyrinae), *Glaucopsyche lygdamus* (Doubleday) and *Plebejus saepiolus* (Boisduval) (Lycaenidae, Polyommatinae), and *Papilio canadensis* Rothschild and Jordan (Papilionidae: Papilioninae). These specimens were previously examined to determine the extent of their genetic isolation and divergence in the Peace River grasslands (Bromilow 2007 Chapter 2). Specimens were collected at 78 localities, predominately in the province of Alberta (Table 2-1, Bromilow 2007 Chapter 2).

Between 12 and 25 specimens were used per species per region (i.e. Peace River region versus southern Alberta and surrounding areas), totalling between 28 and 45 per species (Appendix 1, Bromilow Chapter 2). Whenever possible, an equal number of specimens were used from each region, however this was limited by

availability. Whole genomic DNA was extracted using the QiAMP DNA minikit (Qiagen, Valencia California). A 1420 base-pair section of cytochrome oxidase subunit I (COI) was amplified, generally in two fragments, and sequenced from each specimen using the protocol outlined in Bromilow (2007 Chapter 2). This section corresponds to *Drosophila yakuba* mtDNA base pair numbers 1534 to 2954 (Clary and Wolstenholme 1985), which begins close to the same location as the 'DNA barcoding' region (Hebert *et al.* 2003a, b).

3.2.2 Sequence length and population analyses

Each 1420 bp section was analyzed in its entirety and as a series of divided sections. Fragments were divided into 710 bp halves, 473 bp thirds (the third at the 3' end being 474 bases), and 355 bp quarters for a total of 10 sections (including the total fragment) (Figure 3-1). There were no insertions or deletions to consider, and all alignment was done manually in either PAUP* version 4.0 beta 10 (Swofford 2003) or Arlequin 2.0 (Schneider *et al.* 2000). Variable nucleotide positions were treated as unordered characters. A single state was assigned to each nucleotide; there were no unassigned or missing characters used in this study.

All sequences were designated as either Peace River or southern (no individuals were taken from the intervening boreal region of 300-400 km extent). Some specimens came from regions outside of Alberta (e.g. Montana), however these locations were part of populations contiguous with those in southern Alberta and were designated as such for clarity. In the case of *Papilio machaon*, the northern and

southern grassland populations in Alberta have been described as separate subspecies (*P.m. pikei* in Peace River and *P.m. dodi* in the south) and are referred to as such.

Various population genetic analyses were used to compare differences within and between the two populations. Nested clade phylogeographic analysis (NCPA) was conducted for all species and fragments using Automated Nested Clade Analysis (ANeCA v. 1.0 Panchal 2007, Panchal and Beaumont 2007). The TCS (Clement *et al.* 2004), nesting algorithm (Panchal 2007), and GeoDis (Posada *et al.* 2000) components of ANeCA were used to create haplotype networks, nest the clades, and determine clade significance respectively. Phylogeographic inferences were determined using the GeoDis inference key component of ANeCA (Posada *et al.* 2000). Geographic co-ordinates are the same as those used by Bromilow (2007 Chapter 2), and were determined either in the field with a GPS unit or with Google Earth (2007).

For both *Papilio machaon* and *Coenonympha tullia*, a subset of unusually divergent haplotypes was found in the Peace River region (see Chapter 2 for a more thorough treatment of these haplotypes). One of the two unusual *P.m. pikei* haplotypes and the single anomalous *C. tullia* haplotype were too divergent to be included in NCPA.

Arlequin v. 2.0 (Schneider *et al.* 2000) was used to perform Analysis of Molecular Variance (AMOVA) and associated measures (Fst, variance components), exact tests of sample differentiation and molecular diversity statistics (haplotype diversity and percent sequence divergence). Populations were grouped in the same manner as the nested clade analyses; however all highly divergent haplotypes of *P.*

machaon were included as they composed a substantial number of the specimens of *P.m. pikei*, while the divergent haplotype in *C. tullia* was both included and excluded in separate analyses. Standard deviations for haplotype diversity and percent sequence divergence were calculated within AMOVA using parametric bootstrapping.

Omland *et al.*'s (2006) framework for the characterization of intermediate divergence was applied to all species, however detailed analyses are only shown here for two species that exhibited substantial intermediate divergence between regions. Consensus cladograms of the different fragments were created using unweighted parsimony heuristic searches in PAUP* (Swofford *et al.* 2003). These trees were compared to determine whether intermediate divergence states changed with different sequence lengths/regions.

3.3 Results

Shorter sequences contained fewer variable nucleotide sites, and thereby had fewer numbers of observed haplotypes (Table 3-1). While this affected phylogenetic inferences and population genetic statistics, there was no single section that was consistently similar or dissimilar to the total 1420 bp fragment for all species. The quarters and thirds were variable in their information content (i.e. informative base pairs), and haplotype diversity and sequence divergence often decreased to zero in the Peace River populations of disjunct species.

Sequence length was not the sole determinant of diversity; genetic divergence and phylogeographic inferences varied depending on the region of COI examined. However, no single region of COI consistently had the greatest haplotype diversity (Figure 3-2) or percent sequence divergence (Figure 3-3). The two *Oeneis* species exhibited increased genetic diversity (haplotype diversity and sequence divergence) toward the 3' end of COI while the two *Satyrium* showed decreased diversity toward the 3' end. *Papilio machaon* and *Plebejus saepiolus* exhibited relatively uniform levels of genetic diversity across COI sequence while the remaining species showed variable regions of diversity.

Genetic diversity (haplotype diversity and percent sequence divergence) in the 1420 bp section was variable not only between species but also within species between regions (Figures 3-2, 3-3). Four of the disjunct species exhibited lower genetic diversity in the Peace River region. The exception was *P. machaon*, which exhibited higher genetic diversity due to the presence of a subset of highly divergent haplotypes in *P.m. pikei*. The continuously distributed species exhibited relatively equal divergence between regions, with the exception of *E. epipsodea,* which resembled the disjunct species. Haplotype diversity and percent sequence divergence were overall similar when the sequences were divided into segments. The difference between Peace River and southern populations increased toward the 3' end of COI in the *Oeneis* species, and decreased in the *Satyrium* spp. Other species were more variable; *Papilio machaon* exhibited higher diversity in the Peace River region, while *P. canadensis* and *Plebejus saepiolus* were overall fairly similar between geographic

regions regardless of COI section. Genetic diversity was highly variable between the smallest fragments, becoming increasingly stable as the fragments increased in size.

Clade significance as determined by NCPA was also highly variable between species. Consequently, phylogeographic inferences varied substantially between species (Bromilow Chapter 2). When the sequence was divided into sections, clade significance and phylogeographic inferences varied greatly both within and between species, and no single section of COI reproduced the same clade significance as the total fragment (Table 3-2). *Papilio canadensis* was the only species that was always non-significant, regardless of sequence length, while significance in *Erebia epipsodea* was consistently inconclusive. The other eight species all showed some phylogeographic patterns, but not for all sections. While clade significance was seen in eight species when examining the full 1420 bp fragment, only one of these species (*P. machaon*) showed some clade significance from every fragment, regardless of size and location. The remaining species were all non-significant in at least a third- and quarter-segment. *Plebejus saepiolus* was genetically very diverse, but showed no phylogeographic structure in the largest fragment (Bromilow 2007 Chapter 2). Smaller sections, on the other hand, showed scattered significance.

Fst significance was seen in all 5 disjunct species, as well as *C. tullia* and *E. epipsodea* (Table 3-3). Much like the NCPA data, no single fragment reproduced these results exactly, although the halves and the first third differed only from the 1420 bp fragment in a single taxon (Table 3-3). *Satyrium titus* was the only species with a significant Fst value for all segment sizes and regions. Fst values were never

significant for *P. saepiolus* or *P. canadensis*, and only in one section (the second half) for *G. lygdamus*.

Characterization of intermediate divergence patterns varied between species and was highly dependent on sequence length (results shown only for the two species that provide the most information). *Coenonympha tullia* was the only species that exhibited allophyly from the total sequence (excluding the anomalous haplotype) (Figure 3-4). *Oeneis uhleri* (Figure 3-5) and *Plebejus saepiolus* exhibited allotypy, while all other species exhibited neotypy to varying degrees. The two *Satyrium* species each exhibited a single unique haplotype in the Peace River region, and otherwise only exhibited differences in haplotype frequency. While there are no shared haplotypes in *Plebejus saepiolus*, the lack of population structure suggests that allotypy is in this case due to hyper-variability, and not intermediate divergence (Bromilow Chapter 2). As the fragments decrease in size, allotypy in *O. uhleri* immediately becomes neotypy, and eventually haplotypes only differ in frequency between populations (Figure 3-5). *Coenonympha tullia* presents a more complicated situation; the first and second halves show neotypy and allotypy respectively, while the middle third and third quarter continue to exhibit allophyly (Figure 3-4). Other short fragments were variable, exhibiting allotypy, neotypy, and in one case panmixia. When shorter sequences are examined for the remaining neotypic species, inferences either remain neotypic, or descend to haplotype frequency differences or panmixia.

3.4 Discussion

3.4.1 Fragment size

The ten butterfly species differed in a number of respects, even when examining the entire 1420 bp section of COI (addressed in greater detail by Bromilow Chapter 2). *Coenonympha tullia* exhibited the greatest phylogenetic and phylogeographic structuring of all the butterflies (though this structuring may be unrelated to the Peace River grasslands; more work is needed). The significant population structure and lack of haplotypes shared between regions suggested that this species is not genetically continuous, despite the lack of disjunction in its distribution (Bromilow Chapter 2). The complex phylogenetic and phylogeographic history of *C. tullia* is best detailed by the full 1420 bp section, thereby illustrating the importance of large sequences to the accurate characterization of deep intermediate divergence (i.e. allophyly). *Plebejus saepiolus* exhibited very high genetic diversity, but genetic structure is only seen in some of the smaller fragments. This indicated that structure in genetically variable species can appear significant with a smaller dataset, or that this particular species contains conflicting characters that mask those that trace the true history. Further investigation of this issue in other similarly diverse species would be highly informative.

Percent sequence divergence and haplotype diversity varied greatly between species and fragment lengths. Decreasing sequence length yielded increasingly dissimilar patterns, and smaller sections rarely approximated the diversity of the total

1420 bp section. This emphasizes the importance of examining large fragments of COI to accurately characterize recently diverged populations and elucidate population structure.

Phylogeographic differences between disjunct and continuously distributed species are most apparent when examining the full 1420 bp fragment. If *C. tullia* is considered a disjunct species, then only two phylogeographic inferences are seen amongst the continuously distributed species, and one of these is inconclusive (Table 3-2). By contrast, the disjunct species (including *C. tullia*) exhibit 16 phylogeographically significant clades. Shorter sequences tend to maintain this disparity, although the total number of inferences drops off sharply.

Phylogeographic inferences varied a great deal between fragments of COI, including some instances where genetic diversity did not vary. While quarters (355 bp) demonstrated a good deal of population structure, it is important to note that half of the phylogeographic inferences they exhibited were inconclusive. Shortening sequences increased synonymy and similarity between haplotypes, thereby causing many clades to become non-significant or inconclusive. This also decreased the likelihood of observing allopatric fragmentation, favouring instead the inferences that result from shared haplotypes (i.e. restricted gene flow, contiguous range expansion, and past fragmentation).

Intermediate divergence is best characterized with a large fragment of DNA. The decrease in phylogenetic information that accompanies sequence coNCPAtenation generally lessens the degree of intermediate divergence. The smallest sequences can even cause highly structured populations to appear panmictic

(Figure 3-4). Allotypy and allophyly likely represent considerable genetic isolation in populations (Omland *et al.* 2006), although my data show that these states can easily be underestimated with short DNA sequences. As with other analyses, information content and assertions varied greatly within and between sections. The 710 bp halves, which are of importance to initiatives such as DNA barcoding (Hebert *et al.* 2003a, b), were unable to replicate the divergence seen in the entire fragment.

Increasing sequence length may act as a kind of parallel to the evolution of intermediate divergence, as shallower population fixation is often interpreted when information is lost in shorter sequences. However, this may not be the case if there is a great deal of heterogeneity across the sequence (e.g. *C. tullia*, Figure 3-5). This suggests that the polyphyletic stages of intermediate divergence are correctly nested, which had been questioned by Omland *et al.* (2006).

While genetic variation was regionally consistent within genera, it varied greatly between different butterfly species. Hebert *et al.* (2003b) have suggested that sequence variability is relatively equal between regions of COI, however this finding is not supported in many sister pairs and species groups in both Lepidoptera and Diptera (Roe and Sperling 2007a). The patterns of variation observed in Peace River butterflies suggest that there can be significant differences in information content across COI even at low levels of divergence, and that intermediate divergence of taxa can be difficult to characterize using small (<700bp) fragments of COI. Others have noted the limitations of DNA barcoding when identifying shallow divergences between species (Moritz and Cicero 2004, Sperling 2003, Rubinoff 2006a, Roe and Sperling 2007a).

3.4.2 Fragment Location

No single region of COI can be recommended on the basis of consistent haplotype variation or % sequence divergence. Variation between segments was most apparent in southern Alberta, especially for the disjunct species. This is most plausibly due to bottlenecks that have reduced genetic diversity in the Peace River region and/or the larger geographic sample area in the south. However, this does not suggest that the choice of fragment size or region is irrelevant in isolated species; small fragments (i.e. thirds or smaller) often showed no variation in the Peace River, thereby overemphasizing the genetic bottleneck and/or underemphasizing regional divergence.

Phylogeographic inferences were generally inconsistent between most regions of COI, though there were many of inconclusive clades among all the small segments (thirds and quarters). The final third demonstrated more population structure in the continuously distributed species than disjunct ones, though this is mainly due to the high sequence variation in *Plebejus saepiolus*. Inferred phylogeography varied in many cases where genetic diversity did not. *Papilio machaon* exhibited very different phylogeographic inferences between regions, despite relatively constant genetic and haplotype diversity. Other species exhibited consistent genetic variability between 710 bp halves, but still exhibited very different phylogenetic inferences (notably *C. tullia*, *G. lygdamus* and *P. saepiolus*). Thus even species with relatively

even variation across COI are susceptible to different phylogeographic interpretations based on different lengths and regions of sequence.

Intermediate divergence exhibited a range of states across different regions of COI (Figures 3-4, 3-5). This was most prominent between quarter segments of *C. tullia*, which exhibited four different states of intermediate divergence. The second quarter exhibited the greatest phylogenetic resolution, however the third quarter showed the deepest intermediate divergence (allophyly). This suggests that no single region of COI best characterizes intermediate divergence, and that large fragments should be used to encompass the full range of genetic diversity.

3.4.3 Conservation Interpretations

Continuously distributed species are important to understanding intermediate divergence. They generally exhibit neotypy (or less in small fragments), though it is an open question whether this is the result of genetic isolation. In these cases, shallow intermediate divergence may be the result of inadequate sampling of specimens (i.e. the haplotypes are present in both regions but were not found), or they may be the result of isolation by distance (Bromilow Chapter 2). Regardless of the reason, this suggests that the neotypy seen in some of the disjunct species should be treated cautiously; if it is simply the result of isolation by distance, then neotypy alone may not be a safe measure of disjunction. Larger sample sizes may help to clarify this problem. The continuous species also suggest that diverging populations may not exhibit panmixia at the time of isolation; if a population contains haplotypes

isolated by distance, vicariant divergence of a portion of this population could result in instantaneous neotypy.

The high variability seen across COI indicates that conservation decisions should be based on large fragments of DNA to avoid arbitrary misinterpretations. However, if genetic diversity is known to be high in a small region (e.g. the 5' end of COI in *Satyrium* or the 3' end in *Oeneis*), smaller fragments (e.g. 500-700 bp) may potentially still be used to identify populations of conservation interest. That being said, a larger fragment is more likely to identify unique haplotypes, which may help determine population structure both within and between regions. Moreover, modern sequencing capabilities make it easy to obtain 900 bp of sequence, with little additional effort needed to go beyond 500-700 bp (Gunning *et al.* 2002). Nucleotide divergence is extremely important for detecting cryptic species and identifying relationships between sibling species (Hebert *et al.* 2003a, Tautz *et al.* 2003, Monaghan *et al.* 2006), and the information content can vary greatly across COI (Roe and Sperling 2007a). Ideally, studies of population structure and species delimitation that focus primarily on COI should focus on the entire molecule (~1.5 kb), especially if conservation initiatives are being considered.

The patterns seen in divergent populations before the fixation of monophyletic gene trees could have considerable impact on conservation decisions if intermediate divergence is underestimated or misidentified (DeSalle 2005, Omland *et al.* 2006). Moreover, I recommend caution when characterizing neotypy in disjunct populations, as isolation by distance can also lead to the evolution of regionally restricted haplotypes. If neotypy is seen or expected in disjunct populations, it is advisable to

compare genetic divergence to continuously distributed species, thereby serving as a control.

While they have not been investigated here, multiple genes are well worth investigating (e.g. Roe and Sperling 2007b). Mitochondrial DNA only shows the history of maternal gene flow, and has therefore been controversial in systematics and population genetics (Lipscomb *et al.* 2003, Seberg *et al.* 2003). That being said, some nuclear genes are useful for characterizing shallow and deep divergences (Caterino *et al.* 2000), and may strengthen mitochondrial findings (Moritz 1994, Funk and Omland 2003). However, the sequence-length trends seen in mtDNA are also likely to be present in nuclear genes. Future studies assessing the correlation between nuclear and mitochondrial sequence would do well to examine the effect that increasing sequence length has on characterizing divergence.

Isolated populations are often of conservation importance, and an understanding of their genetic status is vital (Collinge 2000, Spielman *et al.* 2004, DeSalle 2005). Funk and Omland (2003) stressed the importance of combining phylogenetic and phylogeographic methods to assess the history of sister species and populations. The Peace River grassland butterflies indicate that relatively large mitochondrial sequences should be used in these analyses in order to accurately characterize phylogeographic status.

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Table 3-1. Mean sequence variability in different lengths and regions of COI.

	Position (as *D. yakuba*)	Length in bp	Mean no. of variable sites		% variable sites		Mean no. haplotypes	
			PR	SA	PR	SA	PR	SA
Full	1534-2954	1420	10.8	10.9	0.761	0.761	7	8.4
1st half	1534-2244	710	5.1	5.1	0.718	0.718	4.2	5.2
2nd half	2245-2954	710	5.7	5.8	0.803	0.817	3.9	6.1
1st third	1534-1967	433	3.3	2.8	0.762	0.647	3.3	3.5
2nd third	1968-2520	433	4.5	4.9	1.039	1.132	3.6	5.2
3rd third	2521-2954	434	3	3.2	0.691	0.737	2.9	3.9
1st quarter	1534-1888	355	2.7	2.7	0.622	0.622	2.8	3.4
2nd quarter	1889-2244	355	2.4	2.4	0.676	0.676	3	3.3
3rd quarter	2245-2599	355	3.1	3.3	0.873	0.930	2.9	3.8
4th quarter	2600-2954	355	2.6	2.5	0.732	0.704	2.8	3.4

Table 3-2. Inference key results by species and fragment. The letters do not indicate succesive significant haplotypes, but rather the total consecutive phylogeographic inferences made by ANeCA for any significant clades.

	Full	Half 1	Half 2	Third 1	Third 2	Third 3	Quarter 1	Quarter 2	Quarter 3	Quarter 4
O. uhleri	A, B, A, C, D	B, D, A	C, C, A	-	A	C, B	-	B	B	C, B
O. alberta	B	-	B	-	-	-	-	-	-	-
S. titus	D, D	B	D	D	D	-	D	C	D	-
S. liparops	C	B, A	A	A, A	C, A	-	A, A	A	C	-
P. machaon	D, C, C, A	B*, A	C, C, A	D, A	B, A, B	B	A	B*, A	A, A	C, A
C. tullia	D, E, A	A, B, B, A	E, A	B*	E, A	-	B*	B*, A, A	E, A	-
E. epipsodea	A	A	-	A	-	A	A	-	-	-
G. lygdamus	C	C	B	-	-	B	-	-	-	B
P. saepiolus	-	C, A	-	B	B	C, B, A	C, B	-	A, A, A	A
P. canadensis	-	-	-	-	-	-	-	-	-	-

A= Inconclusive
B= Restricted gene flow with isolation by distance (* indicates too few clades to fully resolve)
C= Contiguous range expansion
D= Allopatric fragmentation
E= Long-distance colonization and/or past fragmentation

Table 3-3. Fst significance by species and fragment. Positive signs (+) indicate significance, negative signs (-) indicate non- significance.

	Full	Half 1	Half 2	Third 1	Third 2	Third 3	Quarter 1	Quarter 2	Quarter 3	Quarter 4
O. uhleri	+	+	+	+	+	+	-	+	+	+
O. alberta	+	-	+	-	-	+	-	-	-	+
S. titus	+	+	+	+	+	+	+	+	+	+
S. liparops	+	+	+	+	+	-	+	-	-	-
P. machaon	+	+	+	+	+	-	+	+	+	-
C. tullia	+	+	+	+	+	-	+	+	+	-
E. epipsodea	+	+	+	+	-	-	+	-	-	-
G. lygdamus	-	-	+	-	-	-	-	-	-	-
P. saepiolus	-	-	-	-	-	-	-	-	-	-
P. canadensis	-	-	-	-	-	-	-	-	-	-

Figure 3-1. Location and size of the fragment used in this study and its relation to other commonly used cytochrome oxidase I (COI) sequencing regions. Nucleotide positions are as for *Drosophila yakuba* (Clary and Wolstenholme 1985). Fragments are indicated by black bars and their respective forward and reverse primers at the terminal ends (Folmer et al. 1994, Simon et al. 1994, Sperling et al. 1996, Bromilow 2007 Chapter 2).

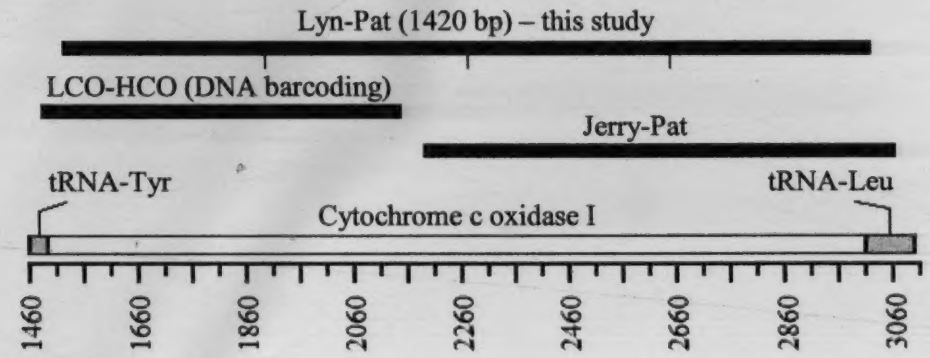


Figure 3-2. Haplotype diversity as a function of fragment length and location. All points represent mean haplotype diversity within a region (Peace River or southern Alberta). Standard deviation is given as vertical error bars. Hollow symbols represent Peace River, while solid symbols represent southern Alberta. Sequence length groups are separated by solid lines and are indicated by letters; F = full (1420 bp), H = half (710 bp), T = third (433-4 bp) and Q = quarters (355 bp). Dashed lines separate the individual sections (e.g. quarters 1-4). Fragments are illustrated in the same order that they occur in the mtDNA (e.g. the half that overlaps with the barcode region is on the left).

Figure 3-2

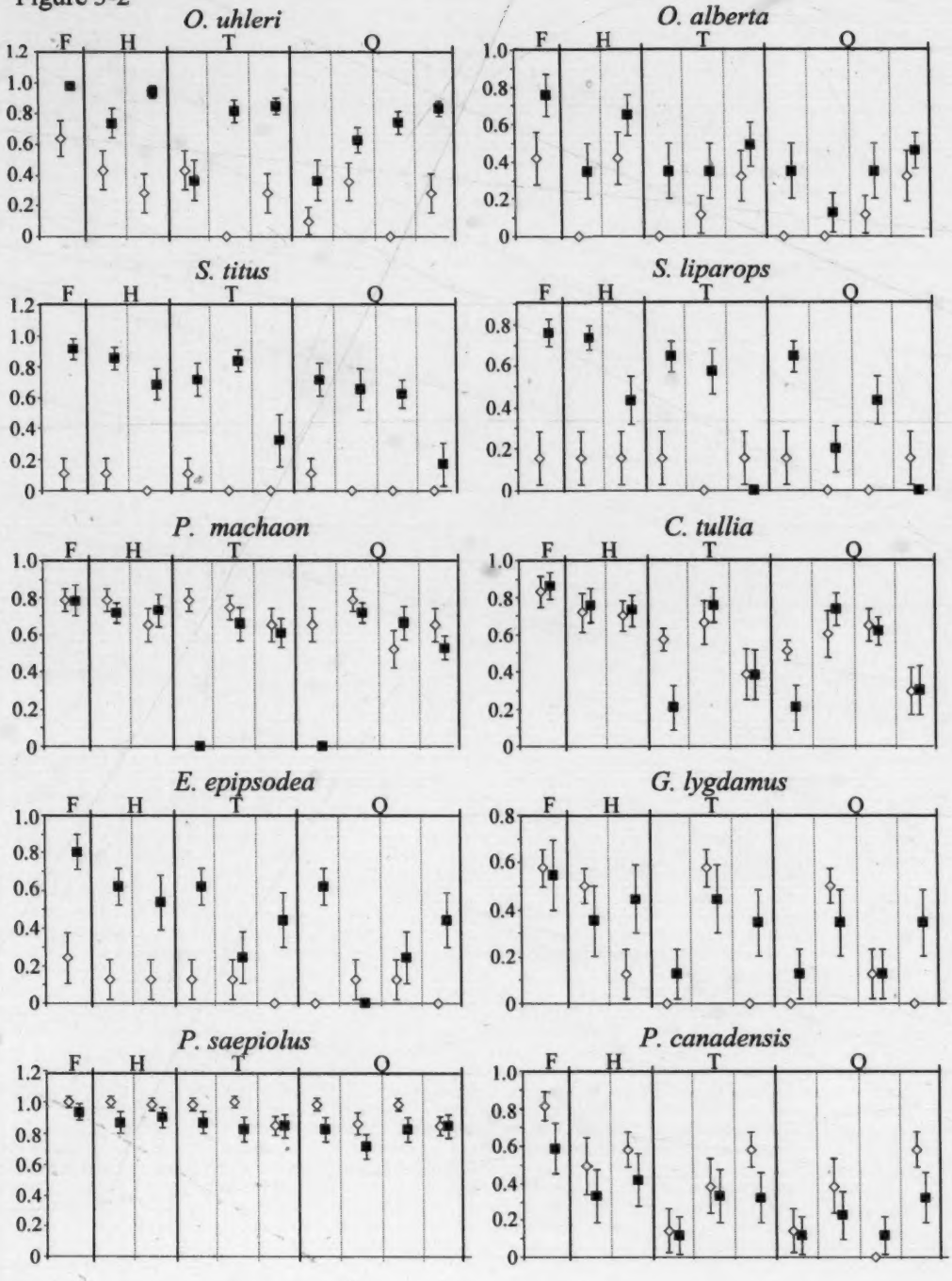

O. uhleri
O. alberta
S. titus
S. liparops
P. machaon
C. tullia
E. epipsodea
G. lygdamus
P. saepiolus
P. canadensis
F
H
T
Q

Figure 3-3. Percent sequence divergence as a function of fragment length and location. All points represent mean percent sequence divergence for each section among all specimens within a region (Peace River or southern Alberta). Standard deviation given is as vertical error bars. Formatting and symbols are as in Figure 3-2.

Figure 3-3

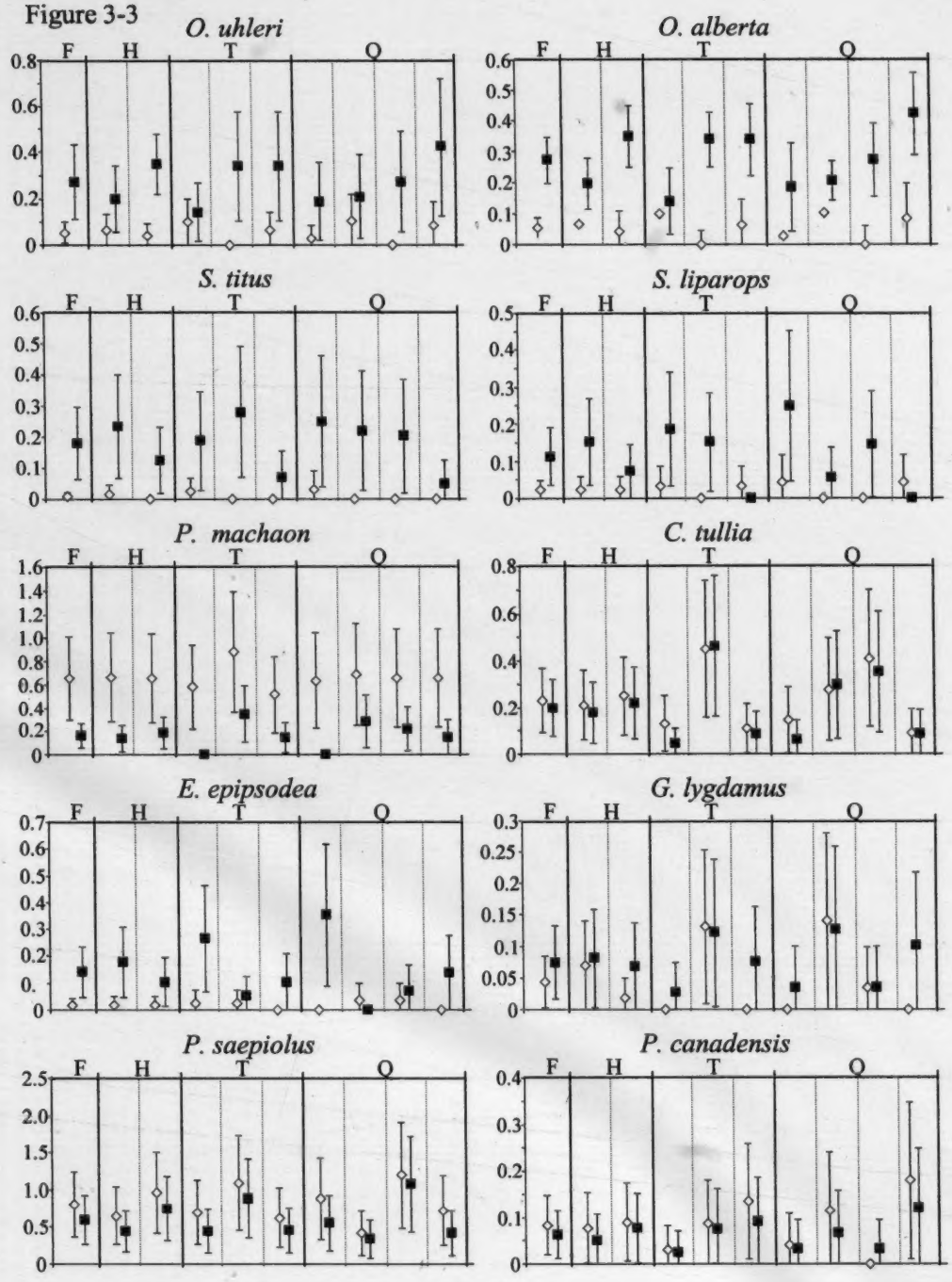

O. uhleri
O. alberta
S. titus
S. liparops
P. machaon
C. tullia
E. epipsodea
G. lygdamus
P. saepiolus
P. canadensis
F
H
T
O

Figure 3-4. Characterization of intermediate divergence in *Coenonympha tullia*. All trees represent unrooted strict-consensus cladograms, with the exception of the 2nd quarter tree, which is a majority rule tree (support values are indicated). Haplotypes indicated by an empty circle are found solely in Peace River, while those marked with a square are shared between Peace River and the south. Unmarked clades are found solely in the south. All clades are a single step (i.e. base-pair) apart unless otherwise marked. Filled dots indicate additional steps between clades. Fragments are illustrated from left to right (e.g. the first third is on the left). Text boxes indicate the stage of intermediate divergence, *sensu* Omland (2006): 'ap' – allophyly, at – allotypy, ne – neotypy, hf – haplotype frequency differences and pm – panmixia.

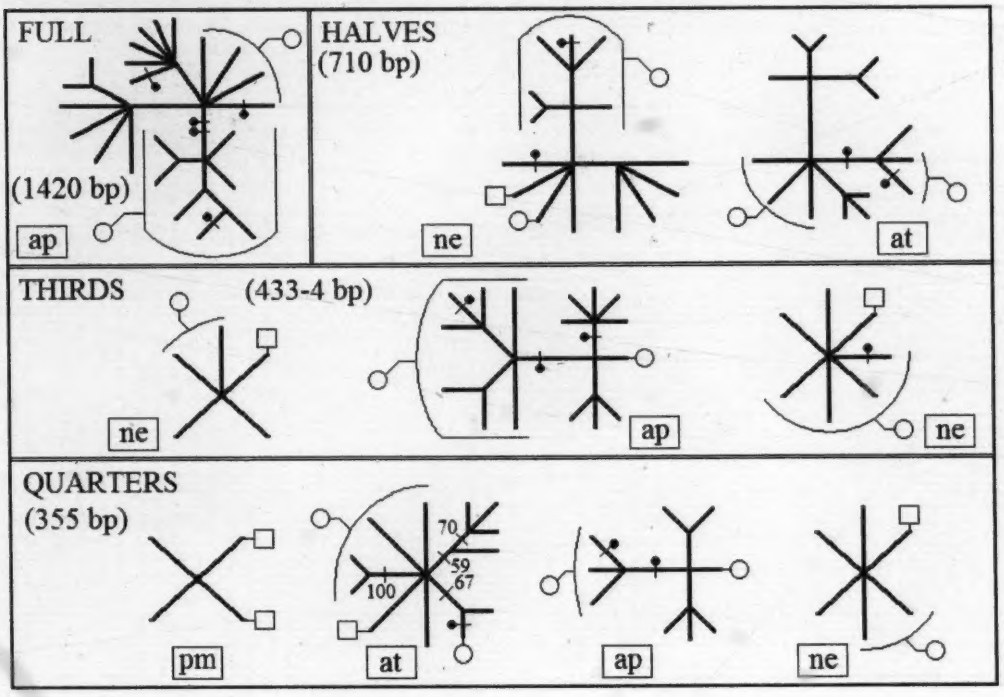
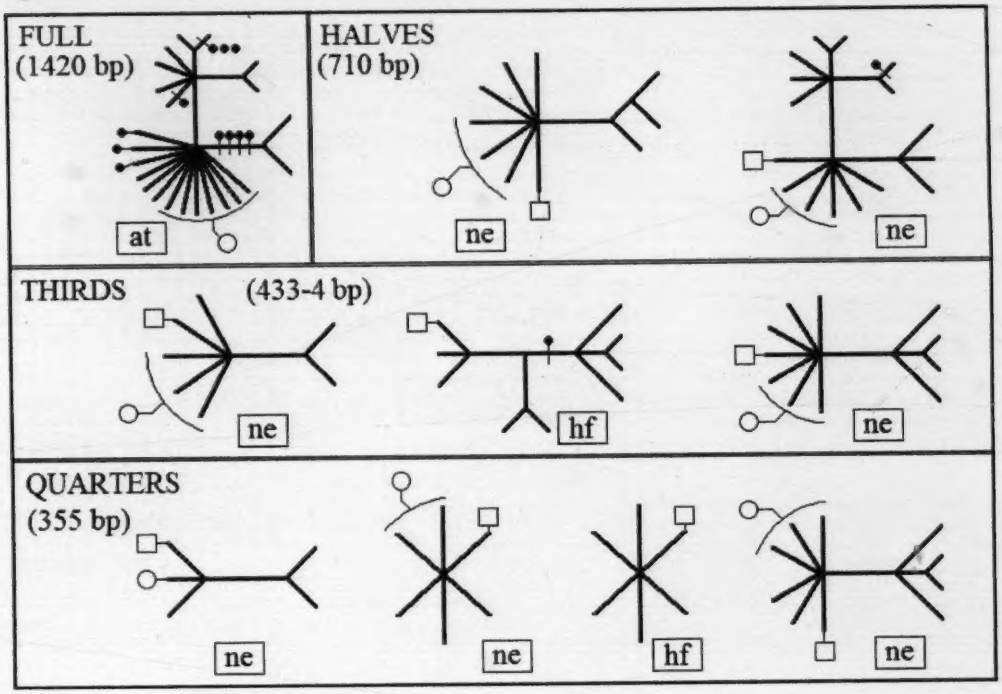


Figure 3-5. Characterization of intermediate divergence in *Oeneis uhleri*. All trees represent unrooted strict-consensus cladograms. Formatting and symbols are as figure 3-4.



Chapter 4 – General discussion and conclusions

This thesis details, for the first time, genetic isolation in several Peace River grassland butterfly species. While the genetic isolation of these insects has implications for their conservation and that of their associated biota, they also provide a model system to distinguish genetic divergence by geographic distance from divergence due to range disjunction. These factors are important when considering the conservation and systematics of any isolated populations.

4.1 Status of the Peace River grassland butterflies

Oeneis uhleri, *O. alberta*, *Satyrium titus*, *S. liparops* and *Papilio machaon pikei* all exhibit some degree of genetic divergence in cytochrome c oxidase subunit I sequences. This reinforces Kondla *et al.*'s (1994) hypothesis that the Peace River populations of these species are distinct from those found further south. *Coenonympha tullia*, despite being distributed fairly continuously throughout the province, also exhibits clear population structure based on COI sequence. On the other hand, levels of genetic divergence were not consistent between species.

Both *Oeneis* species exhibited genetic differentiation in the Peace River region, though this was more pronounced in *Oeneis uhleri*. While this may be due in part to conflicting phylogenetic signal in *O. alberta* (as evinced by the statistical significance in the 1420 bp fragment but not the 1440 bp fragment), this

does not explain the overall paucity of genetic diversity and population structure in comparison to *O. uhleri*.

The two *Satyrium* species exhibited very similar trends, including evidence of a genetic bottleneck in the Peace River region. The difference in haplotype frequency between the two regions suggests that the Peace River grassland butterflies are genetically isolated despite the close similarity in haplotypes.

Overall, *Papilio machaon pikei* appears to be moderately genetically distinct from other subspecies of *Papilio machaon*. However, the presence of shared haplotypes between three of the four examined subspecies suggests that there has been recent gene flow among this group of subspecies. The presence of a subset of highly divergent haplotypes within the Peace River further complicates the issue. This species group has proven biologically complex (see Sperling 1987, McCorkle and Hammond 1989, Sperling 1990, Sperling and Harrison 1994) and these results suggest that extensive sharing of mtDNA may complicate subspecies diagnosis. Further genetic work is needed to resolve these relationships.

The apparent isolation and population structure of *Coenonympha tullia* was unexpected. Kondla *et al.* (1994) suggested that the native-prairie ringlets in Peace River appeared morphologically distinct, however my sampling covered a broad range of habitats, including disturbed and non-native grassland. The presence of a highly divergent haplotype and the high genetic diversity in the

Peace River region indicate that further work is needed to resolve the status of Albertan (and Canadian) *Coenonympha*.

Further genetic and morphological examination of these species is also necessary to determine whether the remaining endemic butterflies of the Peace River grasslands warrant recognition as subspecies, and to determine their placement within larger phylogenies.

4.2 Key Findings

Chapter 2 examined the mtDNA divergence of five Peace River grassland butterfly species in comparison to five continuously distributed species. The variable genetic and phylogeographic results obtained strongly suggest that single-species surveys do not provide an adequate characterization of general faunal endemism and/or divergence for a region. Moreover, the continuously distributed species showed some genetic divergence across the same geographic distance, suggesting that isolation by distance also plays a role in the divergence of the Peace River grassland species. This chapter outlines the first explicit analytical study aimed at discerning isolation across geographic distance from isolation due to population disjunction.

Chapter 3 divided the existing sequence dataset into smaller fragments in order to assess the optimal size and region of COI sequence for use in phylogeographic and population studies. Population genetic metrics and Nested Clade Analysis demonstrated no single most variable region for all species,

although there was some consistency within genera. Moreover, no single region captured the diversity or the same phylogeographic patterns inferred from the total 1420 bp fragment. Inferences also varied greatly when Omland *et al.*'s (2006) framework for characterizing intermediate divergence was applied to the different mtDNA fragments. All of these factors suggest that phylogeographic studies should maximize the region of COI examined. This contrasts with the views espoused by proponents of DNA barcoding (Hebert *et al.* 2003a, b), who maintain that the first 600 bp at the 5' end of COI are sufficient for characterizing divergences within and between species. This finding is extremely important in terms of application; a survey of 250 recently published studies (see below) found 43 that examined COI sequences for phylogeography or population genetics, and these used an average sequence length of 675 bp.

4.3 Methodological discussion

4.3.1 Nested Clade Analysis

Nested Clade Phylogeographic Analysis (NCPA or NCA) allows the user to infer demography from a phylogenetic network. By comparing geographic distance between the locations where haplotypes and clades are found, NCA is able to infer much more detailed genetic structure than Fst based methods such as AMOVA (Templeton *et al.* 1995). However, there is no statistical test built into

NCA analysis to determine the validity of its inferences (Knowles and Maddison 2002).

Despite the discrete temporal isolation of the Peace River grasslands, NCA yielded different phylogeographic hypotheses for several butterflies. Knowles and Maddison (2002) simulated data in which NCA yielded false positives, though Templeton (2004) criticized the dataset as unrealistic. False positives are unlikely in the case of the data presented in this thesis, as other population measures (AMOVA, Mantel test, Exact Test of Sample Differentiation) corroborate the divergence implied by NCA. The Peace River butterflies are almost certainly genetically isolated, although it is unclear what mechanisms have brought about this isolation.

There are several possible explanations for the variation in phylogeographic patterns between the grassland species: 1) the different species do in fact show different demographic histories, 2) stochastic effects, such as early random bottlenecks, have resulted in qualitatively different phylogeographic patterns, 3) the DNA sequence and/or sample size obtained in this study is insufficient to characterize various states of divergence, and 4) NCA inaccurately characterizes populations exhibiting limited genetic divergence. Chapter 2 demonstrated very different levels of genetic diversity between some of the Peace River grassland butterfly species, suggesting that demographic histories may vary between them. Chapter 3 suggests that the first three possibilities play at least some part in the variability of the inferences; as sequence length decreases, phylogeographic inferences vary more. Ultimately, a larger dataset is needed to

disentangle these factors, though future research should also test for demographic discrepancies between species.

4.3.2 Intermediate divergence

Omland *et al.* (2006) first defined the variety of polyphyletic states that precede gene-tree monophyly. Because this system is currently only a year old, there has not been a great deal of application or investigation (but see Johnson *et al.* 2007, Peters *et al.* 2007, Scheffer and Hawthorne 2007). Mitochondrial DNA sequences in Peace River butterflies indicate that characterization of a specific stage of intermediate divergence depends on the length of sequence used. As sequence length decreases, divergence tends to appear shallower (i.e. more recent, although exceptions are found). In this respect, the increase in number of mutations and amount of information arising from greater lengths of DNA may be parallel to the accumulation of divergence within a set length of DNA over time. Distinguishing between these two effects would be an important exercise for conservation genetics.

4.4 Literature review and research context

A growing body of evidence indicates that phylogeographic and phylogenetic inferences can vary between species due to factors such as life history and genetic variation (Lambeck 1997, Funk and Omland 2003, Roberge

and Angelstam 2004, Payne *et al.* 2005, Soltis *et al.* 2006). Recent meta-analyses have demonstrated that even areas that exhibit phylogeographic congruence can vary at certain scales or between taxa (Thompson 1999, Wen 1999, Gillespie and Roderick 2002, Soltis *et al.* 2006).

Nonetheless, the majority of phylogeographic and population genetic studies have continued to focus on single species or species groups based on a survey of phylogeography and population genetic studies from the journal Molecular Ecology (Table 4-1). I examined 250 papers published from January 2006 to August 2007 (including all published-online articles available as of August 10, 2007). Only 10% of the papers I surveyed examined and compared multiple species in different genera, and several of these focused on host/parasite or symbiotic relationships. Some research can be expected to be very taxonomically restricted, however many phylogeographic studies frame their findings in a larger conservation context. If these single-species studies present a biased view of general patterns of divergence or isolation, conservation initiatives could be negatively affected. Future researchers hoping to address the conservation status of a region or population may find it advantageous to include multiple species in their analyses, thereby strengthening any general conclusions about isolation.

Sixty percent of the studies I examined focused on gene sequences alone or in concert with microsatellites (Table 4-2). Moreover, many of these studies focused on relatively short sequences of DNA; average sequence length for all the mitochondrial genes was less than 1000 bp (Table 4-3). Total sequence length

examined varied a great deal, however the majority of studies examined a total sequence length (across all genes) of less than 1000 bp (Figure 4-1). The variable inferences yielded by shorter DNA fragments from the Peace River butterflies suggest that future studies should consistently aim to sequence larger regions.

Some studies focused on multiple methods of analyzing divergence (Table 4-2), including four studies that focused on three or more methods. However, short sequences can still confound combined methods, as they may falsely conflict with other datasets or fail to provide any informative content.

The inclusion of widespread, common species is now relatively frequent in comparative phylogeography (e.g. Ellis *et al.* 2006, Whitely *et al.* 2006, Hughes and Hughes 2007, Pearman and Weber 2007). However, these studies have not used common species to control for isolation across geographic distance. My study is the first to do so explicitly. Of the 250 papers I examined, only two (Whitely *et al.* 2006, Hughes and Hughes 2007) compared multiple focal taxa to one or more common or broadly distributed taxa in different genera, though for different reasons than those outlined in this thesis. These two studies, and the suite of continuously distributed butterflies I examined in the current study, indicate that genetic diversity in continuously distributed or common species can act as an important baseline for understanding divergence and population structure in other species.

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Table 4-1. Focal taxonomic levels from Molecular Ecology studies (2006-August 2007).

Year/Volume	WS	OS	SG	MSWG	MSDG	Total
2007/16	14	75	29	3	10	131
2006/15	7	73	16	9	15	119
TOTAL	21	147	45	12	25	250

Taxonomic level is indicated by a two or four letter abbreviation: WS = within species (e.g. localized populations), OS = one species, SG = species group, MSWG = multiple species within a genus (not comprising a species group), and MSDG = multiple species in different genera. When a paper examined multiple taxonomic levels (e.g. WS, SG), the most inclusive grouping was scored (SG). Phylogeographic and population studies of introduced and/or invasive species are not included as they cannot necessarily be readily compared to other biologically similar species.

Table 4-2. Composition of analyses in Molecular Ecology 2006-2007.

Analysis type	Single	Two data sources		
	Total	Gene sequence	Microsats	AFLP/RFLP
Gene sequence	129	-	-	-
Microsats	55	21	-	-
A/RFLP, RAPD	15	4	2	-
Allozymes	2	2	1	0
ISSR	0	0	0	1
Intron length	0	1	0	0
SSCP/SNP	2	1	0	1
Morphology	0	5	1	0

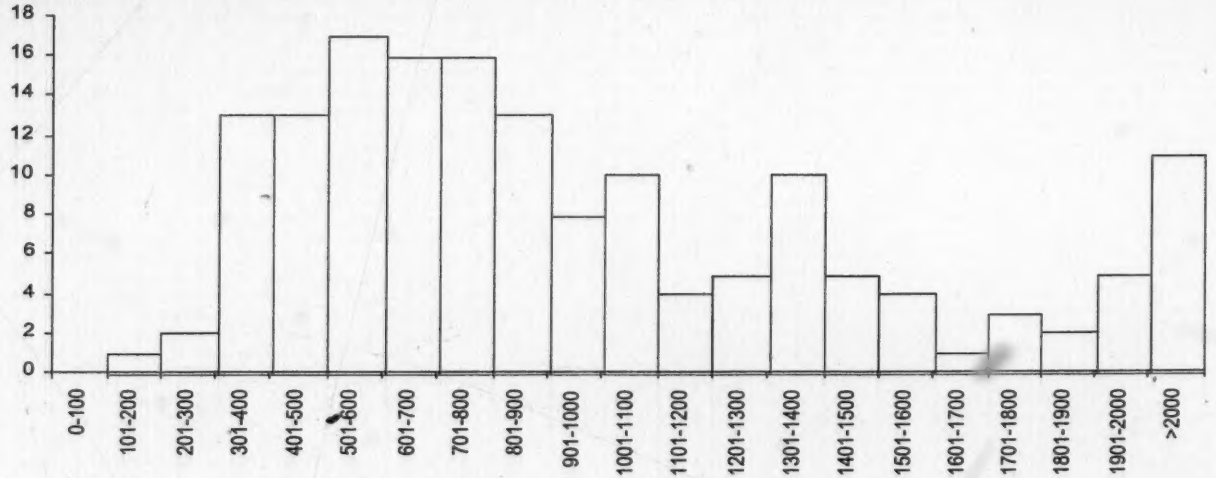
The column 'single' indicates the total number of studies utilizing only the given analytical method, while those using two data sources together are indicated to the right of this. Three meta-analyses and four analyses incorporating more than two data sources are excluded from the table.

Table 4-3. Average length of sequence used by region.

	COI	COII	MtDNA cont. reg.	cytB	ND1,2, 4,6	12s/16s rDNA	CpDNA	Nuclear introns	CNG	ITS	18s/28s rRNA
Number	43	7	50	37	14	12	14	16	32	7	5
Mean length	675	467	553	756	932	482	840	792	690	620	755

Sequence names are given in the first row. Chloroplast DNA (cpDNA), nuclear introns, and coding nuclear genes (CNG) may include multiple genes/sequences. The second row indicates the total number of sequences found in the literature. Note that a single study may have included several of these genes/regions.

Figure 4-1. Total DNA sequence lengths analyzed, categorized by 100 bp increments in studies published in Molecular Ecology 2006-07. Y-axis indicates the number of papers in which the total length of sequence examined falls between the ranges given on the x-axis.



Appendix 1. List of specimens collected by date and locality.

DNA #	Species	Locality	Date Collected	Collector	Date Extracted
1607	*Oeneis uhleri*	Veinerville Hilltops, Medicine Hat, AB	15-May-00	John Acorn	01-May-02
1609	*Oeneis uhleri*	Veinerville Hilltops, Medicine Hat, AB	15-May-00	John Acorn	01-May-02
1610	*Oeneis uhleri*	Kleskun Hill, near Grande Prairie, AB	29-May-00	Margot Hervieux	01-May-02
2201	*Oeneis uhleri*	Moose Mountain Road, AB	07-Jul-01	Felix Sperling	19-May-04
2202	*Oeneis uhleri*	Carlsbad Property, Bear Canyon, Peace River, AB	22-Jun-01	Margot Hervieux	19-May-04
2213	*Oeneis uhleri*	Carbon, AB	18-May-05	Gary Anweiler	18-Jul-05
2214	*Oeneis uhleri*	South of Manyberries, AB	20-May-05	Gary Anweiler	18-Jul-05
2215	*Oeneis uhleri*	South of Manyberries, AB	20-May-05	Gary Anweiler	18-Jul-05
2216	*Oeneis uhleri*	Carbon, AB	18-May-05	Gary Anweiler	18-Jul-05
2217	*Oeneis uhleri*	Carbon, AB	18-May-05	Gary Anweiler	18-Jul-05
2218	*Oeneis uhleri*	Eastsoutheast of Edgerton (7km), AB	27-May-05	Gary Anweiler	18-Jul-05
2219	*Oeneis uhleri*	Road to Green Island, East site (CS1134)	31-May-05	Sean Bromilow and Chris Schmidt	08-Aug-05
2220	*Oeneis uhleri*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	08-Aug-05
2221	*Oeneis uhleri*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	08-Aug-05
2222	*Oeneis uhleri*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	08-Aug-05
2223	*Oeneis uhleri*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	08-Aug-05
2224	*Oeneis uhleri*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	08-Aug-05
2225	*Oeneis uhleri*	Foster's Point, Peace River, AB	22-Jun-01	Adam James	08-Aug-05
2226	*Oeneis uhleri*	Lot 27, Peace River, AB	08-Jun-01	Ed Kolodychuk	08-Aug-05
2227	*Oeneis uhleri*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	08-Aug-05
2228	*Oeneis uhleri*	Highland Park, Peace River, AB	23-Jun-01	Margot Hervieux	08-Aug-05
2230	*Oeneis uhleri*	Cottonwood Park, Lethbridge, AB	23-May-05	Sean Bromilow, Sarah Huang, and Stephane Bourassa	18-Oct-05
2231	*Oeneis uhleri*	Cottonwood Park, Lethbridge, AB	23-May-05	Sean Bromilow, Sarah Huang, and Stephane Bourassa	18-Oct-05
2232	*Oeneis uhleri*	Cottonwood Park, Lethbridge, AB	23-May-05	Sean Bromilow, Sarah Huang, and Stephane Bourassa	18-Oct-05
2233	*Oeneis uhleri*	Cottonwood Park, Lethbridge, AB	23-May-05	Sean Bromilow, Sarah Huang, and	18-Oct-05

				Stephane Bourassa	
2234	*Oeneis uhleri*	Misery Mountain, Peace River, AB (CS1132)	30-May-05	Sean Bromilow and Chris Schmidt	18-Oct-05
2235	*Oeneis uhleri*	Misery Mountain, Peace River, AB (CS1132)	30-May-05	Sean Bromilow and Chris Schmidt	18-Oct-05
2236	*Oeneis uhleri*	Misery Mountain, Peace River, AB (CS1132)	30-May-05	Sean Bromilow and Chris Schmidt	18-Oct-05
2237	*Oeneis uhleri*	Misery Mountain, Peace River, AB (CS1132)	30-May-05	Sean Bromilow and Chris Schmidt	18-Oct-05
2238	*Oeneis uhleri*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	19-Oct-05
2239	*Oeneis uhleri*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	19-Oct-05
2240	*Oeneis uhleri*	Fairview Ski Hill, Peace River region, AB (CS1135)	31-May-05	Sean Bromilow and Chris Schmidt	19-Oct-05
2241	*Oeneis uhleri*	Fairview Ski Hill, Peace River region, AB (CS1135)	31-May-05	Sean Bromilow and Chris Schmidt	19-Oct-05
2847	*Oeneis uhleri*	Chinook Butterfly Circle, AB	04-Jun-00	Barb and Jim Beck	14-Jun-06
2848	*Oeneis uhleri*	Medicine Hat area, near Veinerville, AB	15-May-00	John Acorn	14-Jun-06
2849	*Oeneis uhleri*	Cottonwood Park, Lethbridge, AB	23-May-05	Sean Bromilow, Sarah Huang, and Stephane Bourassa	14-Jun-06
2850	*Oeneis uhleri*	Cottonwood Park, Lethbridge, AB	23-May-05	Sean Bromilow, Sarah Huang, and Stephane Bourassa	14-Jun-06
2851	*Oeneis uhleri*	Dinosaur Provincial Park, AB	21-May-00	Gary Anweiler	14-Jun-06
2852	*Oeneis uhleri*	Crooked Lake, SK	23-May-03	Gary Anweiler	14-Jun-06
2853	*Oeneis uhleri*	North Battleford, SK	22-May-03	Gary Anweiler	14-Jun-06
1608	*Oeneis alberta*	Kleskun Hill, near Grande Prairie	29-May-00	Margot Hervieux	01-May-02
2203	*Oeneis alberta*	Tollman Bridge, Red Deer River Valley, AB	11-May-05	Sean Bromilow and Gary Anweiler	19-May-04
2204	*Oeneis alberta*	12' Davis, Peace River, AB	27-May-05	Margot Hervieux	19-May-04
2205	*Oeneis alberta*	South of Manyberries, AB	20-May-05	Gary Anweiler	18-Jul-05
2206	*Oeneis alberta*	Carbon, AB	18-May-05	Gary Anweiler	18-Jul-05
2207	*Oeneis alberta*	Carbon, AB	18-May-05	Gary Anweiler	18-Jul-05
2208	*Oeneis alberta*	Carbon, AB	18-May-05	Gary Anweiler	18-Jul-05
2209	*Oeneis alberta*	South of Manyberries, AB	20-May-05	Gary Anweiler	18-Jul-05
2210	*Oeneis alberta*	South of Chauvin (12km) AB	27-May-05	Gary Anweiler	18-Jul-05
2211	*Oeneis alberta*	South of Chauvin (12km) AB	27-May-05	Gary Anweiler	18-Jul-05
2212	*Oeneis alberta*	Carbon, AB	18-May-05	Gary Anweiler	18-Jul-05
2824	*Oeneis alberta*	Crooked Lake, SK	24-May-05	Gary Anweiler	14-Apr-06

2825	*Oeneis alberta*	Crooked Lake, SK	24-May-05	Gary Anweiler	14-Apr-06
2832	*Oeneis alberta*	Kleskun Hill, near Grande Prairie, AB (CS1137)	09-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2833	*Oeneis alberta*	Kleskun Hill, near Grande Prairie, AB (CS1137)	09-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2834	*Oeneis alberta*	Kleskun Hill, near Grande Prairie, AB (CS1137)	09-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2835	*Oeneis alberta*	Kleskun Hill, near Grande Prairie, AB (CS1137)	09-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2836	*Oeneis alberta*	Road to Green Island, West site (CS1133)	10-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2837	*Oeneis alberta*	Campsite along Shaftesbury trail, Peace River, AB	10-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2838	*Oeneis alberta*	12' Davis, Peace River, AB	10-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2839	*Oeneis alberta*	12' Davis, Peace River, AB	10-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2840	*Oeneis alberta*	12' Davis, Peace River, AB	10-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2841	*Oeneis alberta*	12' Davis, Peace River, AB	10-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2842	*Oeneis alberta*	12' Davis, Peace River, AB	10-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2843	*Oeneis alberta*	12' Davis, Peace River, AB	10-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2844	*Oeneis alberta*	Misery Mountain, Peace River, AB (CS1132)	07-May-06	Doug Macauley	23-May-06
2845	*Oeneis alberta*	Misery Mountain, Peace River, AB (CS1132)	07-May-06	Doug Macauley	23-May-06
2846	*Oeneis alberta*	Kleskun Hill, near Grande Prairie, AB (CS1137)	21-May-01	Margot Hervieux	14-Jun-06
2854	*Oeneis alberta*	West of Dinosaur Provincial Park, AB	30-Apr-00	John Acorn	14-Jun-06
2855	*Oeneis alberta*	Jenner Bridge area, AB	06-May-06	Jason Dombroskie	14-Jun-06
2856	*Oeneis alberta*	Jenner Bridge area, AB	19-May-06	Gary Anweiler	14-Jun-06
2857	*Oeneis alberta*	Jenner Bridge area, AB	06-May-06	Thomas Simonsen	14-Jun-06
2858	*Oeneis alberta*	Crooked Lake, SK	24-May-03	Gary Anweiler	14-Jun-06
2246	*Satyrium titus*	Kleskun Hill, near Grande Prairie, AB (CS1137)	28-Jul-05	Sean Bromilow	21-Nov-05
2247	*Satyrium titus*	Kleskun Hill, near Grande Prairie, AB (CS1137)	28-Jul-05	Sean Bromilow	21-Nov-05
2248	*Satyrium titus*	12' Davis, Peace River, AB	26-Jul-05	Sean Bromilow	21-Nov-05
2249	*Satyrium titus*	12' Davis, Peace River, AB	26-Jul-05	Sean Bromilow	21-Nov-05
2250	*Satyrium titus*	12' Davis, Peace River, AB	27-Jul-05	Sean Bromilow	21-Nov-05
2251	*Satyrium titus*	12' Davis, Peace River, AB	27-Jul-05	Sean Bromilow	21-Nov-05
2252	*Satyrium titus*	Good Spirit Lake, SK	27-Jul-04	Gary Anweiler	21-Nov-05
2293	*Satyrium titus*	12' Davis, Peace River, AB	21-Jul-02	Bonnie Hood	09-Jan-06

2294	*Satyrium titus*	East of Woking, Peace River region, AB	23-Jul-02	Tara Szkoropa	09-Jan-06
2295	*Satyrium titus*	Kleskun Hill, near Grande Prairie, AB (CS1137)	22-Jul-01	Tara Szkoropa	07-Mar-06
2296	*Satyrium titus*	Highland Park, Peace River, AB	16-Jul-02	Tara Szkoropa	07-Mar-06
2297	*Satyrium titus*	12' Davis, Peace River, AB	10-Jul-02	Bonnie Hood	07-Mar-06
2826	*Satyrium titus*	Pakowki Lake Dunes, AB	21-Jul-05	Gary Anweiler	14-Apr-06
2827	*Satyrium titus*	Northwest of Glenboro, Manitoba	29-Jun-04	Gary Anweiler	14-Apr-06
2874	*Satyrium titus*	Southeast Lethbridge, near Golf Course	25-Jul-06	Sean Bromilow	03-Aug-06
2875	*Satyrium titus*	Popson Park, west of Lethbridge	24-Jul-06	Sean Bromilow	03-Aug-06
2876	*Satyrium titus*	Popson Park, west of Lethbridge	24-Jul-06	Sean Bromilow	03-Aug-06
2877	*Satyrium titus*	Strathcona Science Park, Edmonton, AB	18-Jul-06	Sean Bromilow	03-Aug-06
2878	*Satyrium titus*	Strathcona Science Park, Edmonton, AB	18-Jul-06	Sean Bromilow	03-Aug-06
2879	*Satyrium titus*	Southeast of Drumheller, AB	01-Aug-06	Sean Bromilow	03-Aug-06
2880	*Satyrium titus*	Hilda Sand Dunes, near Medecine Hat, Hwy 41, AB	30-Jul-06	John Acorn	03-Aug-06
2881	*Satyrium titus*	Kleskun Hill, near Grande Prairie, AB (CS1137)	16-Jul-06	Thomas Simonsen	03-Aug-06
3034	*Satyrium titus*	Fort St. John, B.C.	06-Jul-06	Thomas Simonsen	31-Aug-06
3042	*Satyrium titus*	Southeast of Edgerton (2km), AB	24-Aug-02	Gary Anweiler	15-Oct-06
3064	*Satyrium titus*	Dunvegan, Peace River region, AB	19-Jul-03	Felix Sperling	22-Oct-06
3065	*Satyrium titus*	Road to Judah, Peace River, AB (CS1131)	06-Aug-02	Felix Sperling	22-Oct-06
3066	*Satyrium titus*	South of Cleardale, Peace River region prp 054, AB	11-Aug-02	Felix Sperling	22-Oct-06
3067	*Satyrium titus*	Kleskun Hill, near Grande Prairie, AB (CS1137)	21-Jul-03	Felix Sperling	22-Oct-06
3068	*Satyrium titus*	Kleskun Hill, near Grande Prairie, AB (CS1137)	21-Jul-03	Felix Sperling	22-Oct-06
3069	*Satyrium titus*	South Cecil Road, Peace River region, prp 055	11-Aug-02	Felix Sperling	22-Oct-06
3070	*Satyrium titus*	Akasu Hill, near Vegreville, AB	14-Jul-06	Sean Bromilow	22-Oct-06
2253	*Satyrium liparops*	12' Davis, Peace River, AB	12-Jun-04	Bonnie Hood	21-Nov-05
2298	*Satyrium liparops*	Highland Park, Peace River, AB	16-Jul-02	Tara Szkoropa	07-Mar-06
2882	*Satyrium liparops*	Fulton Ravine, Edmonton	30-Jun-06	Gary Anweiler	03-Aug-06
2883	*Satyrium liparops*	Ada Blvd, Edmonton, AB	10-Jul-06	Sean Bromilow	03-Aug-06
2884	*Satyrium liparops*	Ada Blvd, Edmonton, AB	10-Jul-06	Sean Bromilow	03-Aug-06
2885	*Satyrium liparops*	Ada Blvd, Edmonton, AB	10-Jul-06	Sean Bromilow	03-Aug-06

2886	*Satyrium liparops*	Ada Blvd, Edmonton, AB	10-Jul-06	Sean Bromilow	03-Aug-06
2887	*Satyrium liparops*	Ada Blvd, Edmonton, AB	10-Jul-06	Sean Bromilow	03-Aug-06
2888	*Satyrium liparops*	Ada Blvd, Edmonton, AB	16-Jul-06	Sean Bromilow	03-Aug-06
2889	*Satyrium liparops*	Ada Blvd, Edmonton, AB	16-Jul-06	Sean Bromilow	03-Aug-06
2890	*Satyrium liparops*	Helen Schuler Coulee Center, Lethbridge, AB	21-Jul-06	Sean Bromilow	03-Aug-06
2891	*Satyrium liparops*	Helen Schuler Coulee Center, Lethbridge, AB	21-Jul-06	Sean Bromilow	03-Aug-06
2892	*Satyrium liparops*	Helen Schuler Coulee Center, Lethbridge, AB	21-Jul-06	Sean Bromilow	03-Aug-06
2893	*Satyrium liparops*	Popson Park, west of Lethbridge	24-Jul-06	Sean Bromilow	03-Aug-06
2894	*Satyrium liparops*	Popson Park, west of Lethbridge	24-Jul-06	Sean Bromilow	03-Aug-06
3043	*Satyrium liparops*	Akasu Hill, near Vegreville, AB	14-Jul-06	Sean Bromilow	15-Oct-06
3071	*Satyrium liparops*	Wainwright Dunes, AB	13-Jul-02	Doug Macauley	22-Oct-06
3072	*Satyrium liparops*	Wainwright Dunes, AB	13-Jul-02	Doug Macauley	22-Oct-06
3073	*Satyrium liparops*	Edmonton, AB	12-Jul-03	Gary Anweiler	22-Oct-06
3074	*Satyrium liparops*	Edmonton, AB	12-Jul-03	Gary Anweiler	22-Oct-06
3075	*Satyrium liparops*	Edmonton, AB	12-Jul-03	Gary Anweiler	22-Oct-06
3076	*Satyrium liparops*	Edmonton, AB	08-Jul-06	Gary Anweiler	22-Oct-06
3077	*Satyrium liparops*	Dunvegan, Peace River region, AB	19-Jul-03	Felix Sperling	22-Oct-06
3078	*Satyrium liparops*	Dunvegan, Peace River region, AB	19-Jul-03	Felix Sperling	22-Oct-06
3079	*Satyrium liparops*	Dunvegan, Peace River region, AB	19-Jul-03	Felix Sperling	22-Oct-06
3080	*Satyrium liparops*	Highland Park, Peace River, AB	19-Jul-03	Felix Sperling	22-Oct-06
3081	*Satyrium liparops*	Highland Park, Peace River, AB	19-Jul-03	Felix Sperling	22-Oct-06
3082	*Satyrium liparops*	Highland Park, Peace River, AB	19-Jul-03	Felix Sperling	22-Oct-06
3083	*Satyrium liparops*	Highland Park, Peace River, AB	19-Jul-03	Felix Sperling	22-Oct-06
3084	*Satyrium liparops*	Highland Park, Peace River, AB	19-Jul-03	Felix Sperling	22-Oct-06
3085	*Satyrium liparops*	East of Ksituan, AB	17-Jul-02	Denise Kurina and Annette Baker	22-Oct-06
3086	*Satyrium liparops*	12' Davis, Peace River, AB	07-Jul-02	Bonnie Hood	22-Oct-06
3087	*Satyrium liparops*	Highland Park, Peace River, AB	10-Jul-02	Margot Hervieux	22-Oct-06
261	*Papilio machaon pikei*	Taylor, BC		Felix Sperling	56.177
262	*Papilio machaon pikei*	Taylor, BC		Felix Sperling	56.177

263	*Papilio machaon pikei*	Taylor, BC		Felix Sperling	56.177
264	*Papilio machaon pikei*	Taylor, BC		Felix Sperling	56.177
1611	*Papilio machaon pikei*	Fairview Ski Hill, Peace River region	02-Jul-01	Margot Hervieux	10-May-02
2300	*Papilio machaon pikei*	South of Bear Canyon, Peace River region, AB	22-Jun-02	Annette Baker and Tara Szkoropa	07-Mar-06
2801	*Papilio machaon pikei*	Foster's Point, Peace River, AB	20-Jun-01	Adam James	07-Mar-06
2802	*Papilio machaon pikei*	Road to Judah, Peace River, AB (CS1131)	30-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2803	*Papilio machaon pikei*	Highland Park, Peace River, AB	23-Jun-01	Margot Hervieux	07-Mar-06
2804	*Papilio machaon pikei*	Judah Hill Ridgetop, Peace River, AB	11-Jun-01	Adam James	07-Mar-06
2805	*Papilio machaon pikei*	Road to Judah, Peace River, AB (CS1131)	30-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2806	*Papilio machaon pikei*	Peace River townsite, AB	19-Jun-03	Gary Anweiler	07-Mar-06
2807	*Papilio machaon pikei*	Road to Judah, Peace River, AB (CS1131)	30-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
3035	*Papilio machaon pikei*	Highland Park, Peace River, AB	27-Jun-02	Margot Hervieux	31-Aug-06
3308	*Papilio machaon pikei*	Highland Park, Peace River, AB	19-Jul-03	Felix Sperling	30-Jan-07
3309	*Papilio machaon pikei*	Highland Park, Peace River, AB	19-Jul-03	Felix Sperling	30-Jan-07
61	*Papilio machaon dodi*	Drumheller, AB		Felix Sperling	
63	*Papilio machaon dodi*	Circle, McCone Ct, MT		Felix Sperling	
122	*Papilio machaon dodi*	Drumheller, AB		Felix Sperling	
152	*Papilio machaon dodi*	Circle, McCone Ct, MT		Felix Sperling	
154	*Papilio machaon dodi*	Drumheller, AB		Felix Sperling	
155	*Papilio machaon dodi*	Circle, McCone Ct, MT		Felix Sperling	
157	*Papilio machaon dodi*	Circle, McCone Ct, MT		Felix Sperling	
172	*Papilio machaon dodi*	Drumheller, AB		Felix Sperling	
2870	*Papilio machaon dodi*	Jenner Bridge area, AB	06-May-06	Jason Dombroskie	03-Aug-06
2871	*Papilio machaon dodi*	Akasu Hill, near Vegreville, AB	15-Jul-06	Sean Bromilow	03-Aug-06
2872	*Papilio machaon dodi*	Southeast Lethbridge, near Golf Course	25-Jul-06	Sean Bromilow	03-Aug-06
2873	*Papilio machaon dodi*	Helen Schuler Coulee Center, Lethbridge, AB	21-Jul-06	Sean Bromilow	03-Aug-06
3056	*Papilio machaon dodi*	Crowsnest trail, north Lethbridge, AB	22-May-05	Sean Bromilow and Sarah Huang	22-Oct-06
3057	*Papilio machaon dodi*	Crowsnest trail, north Lethbridge, AB	22-May-05	Sean Bromilow and Sarah Huang	22-Oct-06
12	*Papilio machaon aliaska*	Fairbanks, AK		Felix Sperling	

26	*Papilio machaon aliaska*	Fairbanks, AK		Felix Sperling	
203	*Papilio machaon aliaska*	Eagle Summit, AK		Felix Sperling	
223	*Papilio machaon aliaska*	Eagle Summit, AK		Felix Sperling	
260	*Papilio machaon aliaska*	Pink Mtn, BC		Felix Sperling	
2819	*Papilio machaon aliaska*	Pink Mountain, BC	27-Jun-05	Thomas Simonsen	14-Apr-06
2820	*Papilio machaon aliaska*	Pink Mountain, BC	27-Jun-05	Thomas Simonsen	14-Apr-06
77	*Papilio machaon oregonius*	Washington: Thompson Culture		Felix Sperling	
234	*Papilio machaon oregonius*	Klickitat Co., OR		Felix Sperling	
266	*Papilio machaon oregonius*	Klickitat Co., OR		Felix Sperling	
377	*Papilio machaon oregonius*	Wishram, on Columbia River, WA		Felix Sperling	
2244	*Coenonympha tullia*	High Level, AB	18-Jun-03	Gary Anweiler	19-Oct-05
2245	*Coenonympha tullia*	High Level, AB	18-Jun-03	Gary Anweiler	19-Oct-05
2476	*Coenonympha tullia*	Peace River townsite, Peace River region	21-Jun-02	Bonnie Hood	24-Feb-06
2477	*Coenonympha tullia*	Redwood Park, east of Oakland California	19-Jun-98	Felix Sperling	24-Feb-06
2478	*Coenonympha tullia*	Big Creek, University of California Natural Preserve, Big Sur, California	06-Jun-98	Felix Sperling	24-Feb-06
2479	*Coenonympha tullia*	Highland Park, Peace River region, AB	10-Jun-02	Margot Hervieux	24-Feb-06
2480	*Coenonympha tullia*	Bear Canyon, Peace River region, AB	22-Jun-02	Annette Baker	24-Feb-06
2481	*Coenonympha tullia*	Highland Park, Peace River region, AB	09-Jul-02	Margot Hervieux	24-Feb-06
2482	*Coenonympha tullia*	Waterton National Park, AB	10-Jul-05	Thomas Simonsen	24-Feb-06
2483	*Coenonympha tullia*	Waterton National Park, AB	10-Jul-05	Thomas Simonsen	24-Feb-06
2484	*Coenonympha tullia*	Peace River townsite, Peace River region	21-Jun-02	Bonnie Hood	24-Feb-06

2485	*Coenonympha tullia*	Itaska, Pigeon Lake, AB	18-Jun-05	Felix Sperling	24-Feb-06
2486	*Coenonympha tullia*	Bragg Creek, AB	06-Jul-02	Felix Sperling	24-Feb-06
2487	*Coenonympha tullia*	Subalpine area of Moose Mountain Road, AB	06-Jul-03	Felix Sperling	24-Feb-06
2488	*Coenonympha tullia*	Itaska, Pigeon Lake, AB	12-Jul-02	Felix Sperling	24-Feb-06
2489	*Coenonympha tullia*	Dinosaur Provincial Park, AB	07-Jun-00	W. Harrison	24-Feb-06
2490	*Coenonympha tullia*	Base of Moose Mountain Road	07-Jul-01	Felix Sperling	24-Feb-06
2491	*Coenonympha tullia*	Jenner Bridge, AB	19-May-00	Gary Anweiler	24-Feb-06
2492	*Coenonympha tullia*	Chinook Butterfly Circle, AB	04-Jun-00	Barb & Jim Beck	24-Feb-06
2493	*Coenonympha tullia*	Medicine Hat, AB	15-May-00	John Acorn	24-Feb-06
2498	*Coenonympha tullia*	Athabasca, AB	15-Jul-01	Felix Sperling	24-Feb-06
2499	*Coenonympha tullia*	Athabasca, AB	15-Jul-01	Felix Sperling	24-Feb-06
3014	*Coenonympha tullia*	Hillcrest, AB	27-Jun-03	Gary Anweiler	31-Aug-06
3015	*Coenonympha tullia*	Hillcrest, AB	27-Jun-03	Gary Anweiler	31-Aug-06
3016	*Coenonympha tullia*	Helen Schuler Coulee Center, Lethbridge, AB	22-May-05	Sean Bromilow and Sarah Huang	31-Aug-06
3017	*Coenonympha tullia*	Crowsnest trail, north Lethbridge, AB	22-May-05	Sean Bromilow and Sarah Huang	31-Aug-06
3018	*Coenonympha tullia*	Cottonwood Park, Lethbridge, AB	23-May-05	Sean Bromilow, Sarah Huang, and Stephane Bourassa	31-Aug-06
3019	*Coenonympha tullia*	High Level, AB	18-Jun-03	Gary Anweiler	31-Aug-06
3020	*Coenonympha tullia*	High Level, AB	18-Jun-03	Gary Anweiler	31-Aug-06
3021	*Coenonympha tullia*	High Level, AB	18-Jun-03	Gary Anweiler	31-Aug-06
3022	*Coenonympha tullia*	Rigby Site, Peace River region, AB	30-Jun-02	Andrea Pollock	31-Aug-06
3023	*Coenonympha tullia*	Misery Mountain, Peace River, AB (CS1132)	30-May-05	Sean Bromilow and Chris Schmidt	31-Aug-06
3024	*Coenonympha tullia*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	31-Aug-06
3025	*Coenonympha tullia*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	31-Aug-06
3026	*Coenonympha tullia*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	31-Aug-06
3027	*Coenonympha tullia*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	31-Aug-06
3028	*Coenonympha tullia*	Skoropta Site, Peace River, AB	04-Jul-01	Annette Baker	31-Aug-06
3029	*Coenonympha tullia*	Blakley's site, Peace River region, AB	08-Jun-01	Ed Kolodychuk	31-Aug-06
3030	*Coenonympha tullia*	12' Davis, Peace River, AB	07-Jun-01	Adam James	31-Aug-06
3031	*Coenonympha tullia*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	31-Aug-06

3032	*Coenonympha tullia*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	31-Aug-06
3033	*Coenonympha tullia*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	31-Aug-06
3313	*Coenonympha tullia*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	03-Feb-07
3314	*Coenonympha tullia*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	03-Feb-07
3315	*Coenonympha tullia*	Blakley's site, Peace River region, AB	08-Jun-01	Ed Kolodychuk	03-Feb-07
3316	*Coenonympha tullia*	South of Strathcona Science Park, Edmonton, AB	18-Jul-06	Sean Bromilow	03-Feb-07
3317	*Coenonympha tullia*	Akasu Hill, near Vegreville, AB	14-Jul-06	Sean Bromilow	03-Feb-07
2494	*Coenonympha nipisiquit*	Acadian Village Salt Marsh, New Brunswick	14-Aug-05	Felix Sperling	24-Feb-06
2495	*Coenonympha nipisiquit*	Acadian Village Salt Marsh, New Brunswick	14-Aug-05	Felix Sperling	24-Feb-06
2496	*Coenonympha nipisiquit*	Acadian Village Salt Marsh, New Brunswick	14-Aug-05	Felix Sperling	24-Feb-06
2497	*Coenonympha nipisiquit*	Acadian Village Salt Marsh, New Brunswick	14-Aug-05	Felix Sperling	24-Feb-06
1622	*Erebia epipsodea*	Pigeon Lake, AB	02-Jul-01	Felix Sperling	28-May-02
2242	*Erebia epipsodea*	Misery Mountain, Peace River, AB (CS1132)	30-May-05	Sean Bromilow and Chris Schmidt	19-Oct-05
2243	*Erebia epipsodea*	Misery Mountain, Peace River, AB (CS1132)	30-May-05	Sean Bromilow and Chris Schmidt	19-Oct-05
2277	*Erebia epipsodea*	Mackinnon Ravine, Edmonton, AB	24-Jun-00	Felix Sperling	09-Jan-06
2278	*Erebia epipsodea*	Mackinnon Ravine, Edmonton, AB	24-Jun-00	Felix Sperling	09-Jan-06
2279	*Erebia epipsodea*	Airdrie, AB	01-Jul-00	Gary Anweiler	09-Jan-06
2280	*Erebia epipsodea*	Mackinnon Ravine, Edmonton, AB	24-Jun-00	Felix Sperling	09-Jan-06
2281	*Erebia epipsodea*	Pigeon Lake Provincial Park, AB	03-Jul-00	John Acorn	09-Jan-06
2282	*Erebia epipsodea*	12' Davis, Peace River, AB	07-Jun-01	Adam James	09-Jan-06
2283	*Erebia epipsodea*	Blakley's site, Peace River region, AB	08-Jun-01	Ed Kolodychuk	09-Jan-06
2284	*Erebia epipsodea*	12' Davis, Peace River, AB	07-Jun-01	Adam James	09-Jan-06
2285	*Erebia epipsodea*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	09-Jan-06
2286	*Erebia epipsodea*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	09-Jan-06
2287	*Erebia epipsodea*	Blakley's site, Peace River region, AB	08-Jun-01	Ed Kolodychuk	09-Jan-06
2288	*Erebia epipsodea*	Blakley's site, Peace River region, AB	08-Jun-01	Ed Kolodychuk	09-Jan-06

2289	*Erebia epipsodea*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	09-Jan-06
2290	*Erebia epipsodea*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	09-Jan-06
2291	*Erebia epipsodea*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	09-Jan-06
2292	*Erebia epipsodea*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	09-Jan-06
2859	*Erebia epipsodea*	Kleskun Hill, near Grande Prairie, AB (CS1137)	04-Jun-00	Chris Schmidt	14-Jun-06
2860	*Erebia epipsodea*	Pigeon Lake, AB	04-Jun-00	Felix Sperling	14-Jun-06
2861	*Erebia epipsodea*	South of Waterhole, Fairview region, Peace River, AB	20-Jun-02	Darcy Myers	14-Jun-06
2862	*Erebia epipsodea*	Bragg Creek, AB	06-Jul-03	Felix Sperling	14-Jun-06
2863	*Erebia epipsodea*	Bragg Creek, AB	06-Jul-03	Felix Sperling	14-Jun-06
2864	*Erebia epipsodea*	Moose Mountain Road, AB	06-Jul-02	Felix Sperling	14-Jun-06
2865	*Erebia epipsodea*	Moose Mountain Road, AB	07-Jul-01	Felix Sperling	14-Jun-06
2866	*Erebia epipsodea*	Beaver Creek recreation area, Porcupine Hills, AB	05-Jul-01	W.A. Sperling	14-Jun-06
2867	*Erebia epipsodea*	Bragg Creek, AB	07-Jul-01	Felix Sperling	14-Jun-06
2868	*Erebia epipsodea*	Pigeon Lake, Itaska, AB	23-Jun-02	Felix Sperling	14-Jun-06
2869	*Erebia epipsodea*	Pigeon Lake, Itaska, AB	22-Jun-02	Felix Sperling	14-Jun-06
3098	*Erebia epipsodea*	Wildland Park, 8 km south of Peace River, AB	22-May-06	Doug Macauley	19-Dec-06
3318	*Erebia epipsodea*	Halfway up Moose Mountain Road, Bragg Creek, AB	08-Jul-06	Felix Sperling	14-Feb-07
2254	*Glaucopsyche lygdamus*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Sarah Huang	21-Nov-05
2255	*Glaucopsyche lygdamus*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Sarah Huang	21-Nov-05
2257	*Glaucopsyche lygdamus*	Helen Schuler Coulee Center, Lethbridge, AB	22-May-05	Sean Bromilow and Sarah Huang	21-Nov-05
2821	*Glaucopsyche lygdamus*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	14-Apr-06
2822	*Glaucopsyche lygdamus*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	14-Apr-06
2828	*Glaucopsyche lygdamus*	Fairview Ski Hill, Peace River region, AB (CS1135)	11-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2829	*Glaucopsyche lygdamus*	Fairview Ski Hill, Peace River region, AB (CS1135)	11-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2830	*Glaucopsyche lygdamus*	Kleskun Hill, near Grande Prairie, AB (CS1137)	09-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
2831	*Glaucopsyche lygdamus*	12' Davis, Peace River, AB	10-May-06	Sean Bromilow and Jason Dombroskie	23-May-06
3044	*Glaucopsyche lygdamus*	Mackinnon Ravine, Edmonton, AB	24-Jun-00	Felix Sperling	15-Oct-06
3045	*Glaucopsyche lygdamus*	Moose Mountain Road, AB	08-Jul-01	Felix Sperling	15-Oct-06

3046	*Glaucopsyche lygdamus*	Cadomin Cave Trail, Cadomin, AB	04-Jul-02	Felix Sperling	15-Oct-06
3047	*Glaucopsyche lygdamus*	Cadomin Cave Trail, Cadomin, AB	04-Jul-02	Felix Sperling	15-Oct-06
3048	*Glaucopsyche lygdamus*	Fish Butte, Bragg Creek, AB	08-Jul-06	Felix Sperling	15-Oct-06
3049	*Glaucopsyche lygdamus*	Fish Butte, Bragg Creek, AB	08-Jul-06	Felix Sperling	15-Oct-06
3050	*Glaucopsyche lygdamus*	Fish Butte, Bragg Creek, AB	08-Jul-06	Felix Sperling	15-Oct-06
3051	*Glaucopsyche lygdamus*	Itaska, Pigeon Lake, AB	02-Jul-06	Felix Sperling	15-Oct-06
3058	*Glaucopsyche lygdamus*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	22-Oct-06
3059	*Glaucopsyche lygdamus*	Blakley's site, Peace River region, AB	08-Jun-01	Ed Kolodychuk	22-Oct-06
3060	*Glaucopsyche lygdamus*	Lac Cardinal Point, Peace River region, AB	06-Jun-01	Ed Kolodychuk	22-Oct-06
3061	*Glaucopsyche lygdamus*	Mackinnon Ravine, Edmonton, AB	24-Jun-00	Felix Sperling	22-Oct-06
3062	*Glaucopsyche lygdamus*	Pigeon Lake, AB	02-Jul-01	Felix Sperling	22-Oct-06
3063	*Glaucopsyche lygdamus*	Bragg Creek, AB	07-Jul-00	Felix Sperling	22-Oct-06
3100	*Glaucopsyche lygdamus*	Tangent Park, Peace River region, AB	03-Jul-06	Doug Macauley	30-Jan-07
3301	*Glaucopsyche lygdamus*	12' Davis, Peace River, AB	15-May-06	Sean Bromilow and Jason Dombroskie	30-Jan-07
3302	*Glaucopsyche lygdamus*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	30-Jan-07
3303	*Glaucopsyche lygdamus*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	30-Jan-07
3304	*Glaucopsyche lygdamus*	Itaska, Pigeon Lake, AB	04-Jun-06	Felix Sperling	30-Jan-07
3305	*Glaucopsyche lygdamus*	Itaska, Pigeon Lake, AB	04-Jun-06	Felix Sperling	30-Jan-07
3306	*Glaucopsyche lygdamus*	Itaska, Pigeon Lake, AB	04-Jun-06	Felix Sperling	30-Jan-07
3307	*Glaucopsyche lygdamus*	Top of Moose Mountain Road, Bragg Creek, AB	07-Jul-01	Felix Sperling	30-Jan-07
2823	*Plebejus saepiolus*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	14-Apr-06
2896	*Plebejus saepiolus*	Dunvegan, Peace River region, AB	19-Jul-03	Felix Sperling	10-Aug-06
2897	*Plebejus saepiolus*	Misery Mountain, Peace River, AB (CS1132)	30-May-05	Sean Bromilow and Chris Schmidt	10-Aug-06
2898	*Plebejus saepiolus*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	10-Aug-06
2899	*Plebejus saepiolus*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	10-Aug-06
2900	*Plebejus saepiolus*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	10-Aug-06
3001	*Plebejus saepiolus*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	10-Aug-06
3002	*Plebejus saepiolus*	Fairview Ski Hill, Peace River region, AB (CS1135)	31-May-05	Sean Bromilow and Chris Schmidt	10-Aug-06
3003	*Plebejus saepiolus*	Fairview Ski Hill, Peace River region, AB (CS1135)	31-May-05	Sean Bromilow and Chris Schmidt	10-Aug-06

3004	*Plebejus saepiolus*	Moose Mountain Road, AB	08-Jul-00	Felix Sperling	10-Aug-06
3005	*Plebejus saepiolus*	Itaska roadside, Pigeon Lake, AB	22-Jun-00	Felix Sperling	10-Aug-06
3006	*Plebejus saepiolus*	Bragg Creek, AB	08-Jul-00	Felix Sperling	10-Aug-06
3007	*Plebejus saepiolus*	Bragg Creek, AB	07-Jul-01	Felix Sperling	10-Aug-06
3008	*Plebejus saepiolus*	Bragg Creek, AB	07-Jul-01	Felix Sperling	10-Aug-06
3009	*Plebejus saepiolus*	North of Waiparous, AB	07-Jul-03	Felix Sperling	10-Aug-06
3010	*Plebejus saepiolus*	Fish Butte, Bragg Creek, AB	08-Jul-06	Felix Sperling	10-Aug-06
3011	*Plebejus saepiolus*	Fish Butte, Bragg Creek, AB	08-Jul-06	Felix Sperling	10-Aug-06
3088	*Plebejus saepiolus*	Tangent Park, Peace River region, AB	03-Jul-06	Doug Macauley	19-Dec-06
3089	*Plebejus saepiolus*	Tangent Park, Peace River region, AB	03-Jul-06	Doug Macauley	19-Dec-06
3090	*Plebejus saepiolus*	Tangent Park, Peace River region, AB	03-Jul-06	Doug Macauley	19-Dec-06
3091	*Plebejus saepiolus*	Elnora AB	01-Jul-06	Felix Sperling	19-Dec-06
3092	*Plebejus saepiolus*	Elnora AB	01-Jul-06	Felix Sperling	19-Dec-06
3093	*Plebejus saepiolus*	Pigeon Lake, junction of Hwy 771 and 616	02-Jul-06	Felix Sperling	19-Dec-06
3094	*Plebejus saepiolus*	Pigeon Lake, junction of Hwy 771 and 616	02-Jul-06	Felix Sperling	19-Dec-06
3095	*Plebejus saepiolus*	Dunvegan, Peace River region, AB	11-Jun-05	Doug Macauley	19-Dec-06
3096	*Plebejus saepiolus*	Dunvegan, Peace River region, AB	11-Jun-05	Doug Macauley	19-Dec-06
3310	*Plebejus saepiolus*	Lookout on Moose Mountain Road, Bragg Creek, AB	07-Jul-01	Felix Sperling	03-Feb-07
3311	*Plebejus saepiolus*	Lookout on Moose Mountain Road, Bragg Creek, AB	07-Jul-01	Felix Sperling	03-Feb-07
241	*Papilio canadensis*	Bragg Creek, AB		Felix Sperling	
268	*Papilio canadensis*	Fairbanks, AK		Felix Sperling	
304	*Papilio canadensis*	Bragg Creek, AB		Felix Sperling	
306	*Papilio canadensis*	Fairbanks, AK		Felix Sperling	
324	*Papilio canadensis*	Fairbanks, AK		Felix Sperling	
1612	*Papilio canadensis*	Lac Cardinal Point, Peace River region	06-Jun-01	Ed Kolodychuk	10-May-02
1613	*Papilio canadensis*	Lac Cardinal Point, Peace River region	06-Jun-01	Ed Kolodychuk	10-May-02
1614	*Papilio canadensis*	Kleskun Hill, near Grande Prairie, AB	04-Jun-00	B.C. Schmidt	10-May-02
1615	*Papilio canadensis*	Pigeon Lake, AB	02-Jul-01	Felix Sperling	10-May-02

1616	*Papilio canadensis*	Pigeon Lake, AB	02-Jul-01	Felix Sperling	10-May-02
1617	*Papilio canadensis*	Porcupine Hills, Beaver Creek, AB	05-Jul-01	W.A. Sperling	10-May-02
2808	*Papilio canadensis*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2809	*Papilio canadensis*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2810	*Papilio canadensis*	12' Davis, Peace River, AB	30-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2811	*Papilio canadensis*	Road to Judah, Peace River, AB (CS1131)	30-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2812	*Papilio canadensis*	Misery Mountain, Peace River, AB (CS1132)	30-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2813	*Papilio canadensis*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2814	*Papilio canadensis*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2815	*Papilio canadensis*	Road to Green Island, West site (CS1133)	31-May-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2816	*Papilio canadensis*	Kleskun Hill, near Grande Prairie, AB (CS1137)	01-Jun-05	Sean Bromilow and Chris Schmidt	07-Mar-06
2817	*Papilio canadensis*	Peace Point, Peace River region, AB	20-Jun-01	Adam James	07-Mar-06
2818	*Papilio canadensis*	Lac Cardinal Point, Peace River region, AB	06-Jun-01	Ed Kolodychuk	07-Mar-06
3012	*Papilio canadensis*	Fish Butte, Bragg Creek, AB	08-Jul-06	Felix Sperling	10-Aug-06
3013	*Papilio canadensis*	Fish Butte, Bragg Creek, AB	08-Jul-06	Felix Sperling	10-Aug-06
3037	*Papilio canadensis*	Bragg Creek, AB	07-Jul-01	Felix Sperling	31-Aug-06
3038	*Papilio canadensis*	South of Scotswood, Peace River region, AB	12-Jun-02	Tara Szkoropa, Denise Kurina, and Annette Baker	15-Oct-06
3039	*Papilio canadensis*	Bragg Creek, AB	07-Jul-01	Felix Sperling	15-Oct-06
3040	*Papilio canadensis*	Fish Butte, Bragg Creek, AB	08-Jul-06	Felix Sperling	15-Oct-06
3041	*Papilio canadensis*	Fish Butte, Bragg Creek, AB	08-Jul-06	Felix Sperling	15-Oct-06
3052	*Papilio canadensis*	Bragg Creek, AB	08-Jul-01	W. Sperling	22-Oct-06
3053	*Papilio canadensis*	Bragg Creek, AB	08-Jul-00	Felix Sperling	22-Oct-06
3054	*Papilio canadensis*	Upper lookout, Moose Mountain Road, AB	06-Jul-02	Felix Sperling	22-Oct-06
3055	*Papilio canadensis*	Pigeon Lake Inlet Area, AB	01-Jul-03	Felix Sperling	22-Oct-06

Biography

I was born on October 3rd, 1982 in Edmonton, Alberta. I spent the first two years of my life in Edmonton, before moving to the nearby community of St. Albert. My formative years were very happy, and I quickly developed an interest in all things natural. My parents were always happy to support my burgeoning interest in science by finding me countless dinosaur books, traveling out of town to the nearest wildlife sanctuary or wooded area, and listening with remarkable patience while I waxed poetic about the various bugs, birds and dinosaurs with which I had most recently become acquainted.

School was always an interesting time for me. While I was always interested in learning new things, I was constantly reprimanded for talking too much. To this day, I will gladly engage the nearest interested party on whatever topic has most recently piqued my curiosity. School did expose me to new and exciting aspects of biology, and at around 13 years of age, my interests wandered heavily to entomology.

I spent all of high school with the overwhelming desire to get to University and study entomology. Thanks to several very supportive and enthusiastic teachers and friends, I found my way to the University of Alberta.

My undergraduate degree was one of the most eventful periods in my life. While I was now living my buggy dream, I had to decide exactly what kind of entomology to do. I enrolled in Insect Taxonomy with Dr. Felix Sperling, and immediately developed a passion for systematics and evolutionary biology. My fourth year found me enrolled in an undergraduate honours project with Dr. Heather

Proctor and a literature survey course with Dr. Sperling. I was hooked. I applied to graduate studies at the U of A.

Graduate school has been, without a doubt, the most academically and intellectually challenging time of my life, and I have relished every minute. I've discovered an endless world of questions just waiting to be answered, and I plan to keep looking for the most interesting ones. I would never have made it through without the steadying advice of Dr. Sperling, the support (both emotional and financial) of my family, and the many friends I brought with me and made along the way.

My thirst for knowledge has brought me many things, but none more precious than my fiancée Sarah, whom I met in a fourth year science elective. The greatest answers come to questions that are not even asked.